CFTRI LIBRARY
B126

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
LIBRARY
MYSORE.

7

This is from:—
POPULAR BOOK DEPOT,
BOMBAY 7.

THE NEWER KNOWLEDGE OF NUTRITION

THE MACMILLAN COMPANY
NEW YORK · BOSTON · CHICAGO · DALLAS
ATLANTA · SAN FRANCISCO

MACMILLAN AND CO., LIMITED
LONDON · BOMBAY · CALCUTTA · MADRAS
MELBOURNE

THE MACMILLAN COMPANY
OF CANADA, LIMITED
TORONTO

HE NEWER KNOWLEDGE OF NUTRITION

BY

E. V. McCOLLUM, PH.D., SC.D., LL.D.
Professor of Biochemistry, School of Hygiene and Public Health,
The Johns Hopkins University,

ELSA ORENT-KEILES, SC.D.
Associate in Biochemistry, School of Hygiene and Public Health,
The Johns Hopkins University,

AND

HARRY G. DAY, SC.D.
Associate in Biochemistry, School of Hygiene and Public Health,
The Johns Hopkins University.

ILLUSTRATED

Fifth Edition
Entirely Rewritten

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
LIBRARY
MYSORE

NEW YORK
THE MACMILLAN COMPANY

COPYRIGHT, 1918, 1922, 1925, 1929, 1939
BY THE MACMILLAN COMPANY.

All rights reserved—no part of this book may be reproduced in any form without permission in writing from the publisher, except by a reviewer who wishes to quote brief passages in connection with a review written for inclusion in magazine or newspaper.

Set up and electrotyped. Published October, 1918.
Second Edition Entirely Rewritten, April, 1922.
Third Edition Entirely Rewritten, June, 1925.
Fourth Edition Entirely Rewritten, October, 1929.
Fifth Edition Entirely Rewritten, March, 1939.
Fifth Printing, December, 1943.
Sixth Printing, December, 1944.
Seventh Printing, ay, 1946.

126

L; 32 N46

CFTRI-MYSORE
126
Never knowledge

PRINTED IN THE UNITED STATES OF AMERICA

TO

RUSSELL H. CHITTENDEN

Founder of the First Laboratory of Physiological Chemistry in America, Distinguished Investigator, and Inspiring Teacher.

PREFACE TO THE FIFTH EDITION

HE fourth edition of The Newer Knowledge of Nutrition appeared in 1929 and has been out of print since 1935. The book has been completely rewritten, but the plan of the present edition is essentially the same as that of the previous editions. It represents an attempt to interpret the meaning of the extensive experimental work relating to nutrition and described in numerous journals during recent years. Our purpose has been to present the subject matter in a form intelligible and useful to the large number of students, teachers, physicians, dentists, and others who are interested in being informed on the present knowledge of nutrition.

In order to place the modern viewpoints in their proper setting the first two chapters are essentially historical and trace the development of modern concepts of nutrition, only the publications which constitute distinct advances being mentioned. In other chapters, dealing with the several known nutrients, only such references are made to the older literature as are necessary for making clear their importance as foundations for the more recent researches. But an attempt has been made to include all of the essential information about each nutrient, thus making the volume useful as a textbook. In presenting the data from many sources the title, The Newer Knowledge of Nutrition, has been kept in mind. The facts and opinions in what appear to be the more significant original publications have been recorded as nearly as possible in the form of a presentation of the conclusions of the investigators and the type of experimental procedure employed, only a minimum of data being presented which illustrate the basis for conclusions. Where conflicting views are supported by experimental evidence these are cited, and where critical remarks seem justified these are included. A careful re-examination was made by the authors of all the literature which appeared to be significant.

An attempt has been made to prepare a concise survey of the field of nutrition which is more extensive and more inclusive than any other now available and which is kept close to the scientific literature by means of citations from carefully selected references for the benefit of those who may care to extend their reading in the original literature. It is hoped that the book will meet the needs of all who are, like the authors, seeking to determine what is sound and what is speculation

or misinformation in respect to the nutritive needs of several speci
including man; the dietary properties of foodstuffs; the characteriz
tion of malnutrition due to specific or multiple deficiency states; t
occurrence of various types of malnutrition in man and animals
different parts of the world; and the means of dealing effective
with these nutritional problems.

E. V. McCollum
Elsa Orent-Keiles
Harry G. Day

Baltimore, Md.
August, 1938.

CONTENTS

CHAPTER		PAGE
I	The Science of Nutrition, Early History and Development of Concepts	1
II	Nutritive Requirements of the Body, the Modern Concept of Dietary Essentials	15
III	Carbohydrates in Nutrition	32
IV	Lipids in Nutrition	55
V	General Nature of Proteins and Amino Acids and Their Significance in Nutrition	78
VI	Nutritional Value of Proteins and the Effects of Variations in the Level of Dietary Protein	120
VII	Calcium, Phosphorus, and Magnesium	155
VIII	Sodium, Potassium, and Chlorine	192
IX	Iron, Copper, and Nutritional Anemias	211
X	Iodine and Its Relation to Thyroid Function	244
XI	"Trace" Inorganic Elements	261
XII	Chemical Nature of Vitamin A	290
XIII	Nutritional Significance of Vitamin A	308
XIV	Chemical Nature of Vitamin D	336
XV	Significance of Vitamin D in Rickets and Related Diseases	363
XVI	Chemical Nature of Ascorbic Acid (Vitamin C)	398
XVII	Nutritional Significance of Ascorbic Acid (Vitamin C)	417
XVIII	Chemical Nature of Thiamin (Vitamin B_1)	441
XIX	Nutritional Significance of Thiamin (Vitamin B_1)	459
XX	Riboflavin	483
XXI	Pellagra and Animal Deficiency Diseases Related to Pellagra	499
XXII	Other Vitamin Factors	518
XXIII	Vitamin E	532
XXIV	Dietary Properties of Foodstuffs	552
XXV	Appetite, Normal and Perverted	566
XXVI	Dietary Habits of Man in Different Parts of the World	580
XXVII	Diet in Relation to the Teeth	600
XXVIII	Diet in Relation to Healthful Longevity	632
	Appendix	639
	Index	685

CHAPTER

I

The Science of Nutrition, Early History and Development of Concepts

RIMITIVE MAN appears nowhere to have given much thought to e nature of the processes of nutrition. However, he exhibited much ought and foresight in providing a food supply, for throughout uman history food has been one of man's most pressing problems. his is reflected by the biblical maxim, "In the sweat of thy face shalt ou eat bread." The ancient hunter ate the heart of his adversary in e belief that this gave him strength and courage. This appears to ave been the earliest distinction of special nutritive quality in food. rimitive peoples experimented with everything available in the way f food. Long before the dawn of history, the chewer of the coca leaf South America, the devotees of kat in the near East, and the bled lotus eaters of the Orient, had discovered that these vegetable roducts would allay pain and act as stimulants or produce dreamy ntentment. The biblical story of Noah's misadventure with wine of e grape illustrates the early experience with alcohol and showed that is beverage could cheer and give respite from care, although it rought the blush of shame to the cheeks of his modest family.

With the development of a relatively stable society the more favored dividuals acquired a keen appreciation of the appetizing qualities f certain foods and beverages. The earliest extensive account of the ews of the Greeks and Romans concerning the merits of all the more tractive foods available in the Mediterranean region is that of thenaeus, written about 200 A. D., which is a record of the conversa- on about foods and drinks by a company of gentlemen at a banquet. lso it includes their views about the merits of waters from various ources.

From time immemorial mothers must have given much thought to oods which were suitable for young children. It is stated by Chief

Standing Bear ('33) of the Western Sioux Indians, that grandmoth believed a cake, made of a meal composed of dried meat and dr chokeberries, and held together by the fat skimmed from the boi bones of the buffalo, was especially good for young children j beginning to eat solid food. The Sioux were in great measure carnivorous people.

Diodorus (1st C. B.C.) tells of the understanding by the Anci Egyptians of the fact that many diseases were caused by excess eating, and that they employed emetics and aperients to relieve t body (Barach, '28). Much as they knew about several arts such as met lurgy, dyeing, soap, glass making, alloys, amalgams, and medicin they knew scarcely anything of the nature of foods or the processes nutrition.

The concept of four elements, fire, air, earth, and water, int duced by Empedocles (504-432 B.C.), was inadequate for progress an understanding of the nature of nutrition. Hippocrates (460-3 B.C.) insisted that those who attempted to speak on medicine, usi only the concepts of hot or cold, and moist or dry, were clearly m taken. He pointed out that a man who eats raw wheat from t threshing floor, raw meat, and water, suffers severely in his bowe and does not live long, whereas if he eats bread, boiled meat and tak wine, he is well nourished. He observed that some could not eat chee without distress, whereas others with whom it agreed gained from wonderful strength. One of his aphorisms is translated: "Growin bodies have the most innate heat; they therefore require the most foo for otherwise their bodies are wasted. In old people the heat is feeb and they require little fuel, as it were, to the flame, for it would extinguished by much." Modern calorimetry is foreshadowed in th aphorism. Hippocrates also noted that very fat people are likely to d earlier than those who are slender. In his system of medicine, dietin exercise, exposure to sunshine, and psychic diversions were prominen

Erasistratus (310-250 B.C.) apparently performed the first exper ment in nutrition (Garrison, '29). Believing that the pneuma of th air was transformed into spirit in the body, he placed fowls in a j and weighed them and their excreta before and after feeding. Th was the beginning of respiration studies. Galen (130-200 A.D.) studie the digestion of hogs and stated that the stomach was the place which food was resolved into particles small enough to be absorbe (Walsh, '27). Barach (l.c.) states that about 229 A.D. a Chinese o server noted that the urine of a diabetic was so sweet that dogs dran it. By the sixth century the Hindus had records that "honey urin was caused by excessive eating of rice, flour, and sugar.

Leonardo da Vinci (1452-1519) discerned that the body of a animal constantly dies and is constantly born again (Herzfeld, '26

at nourishment can only enter into places where past nourishment expired; that if one restores as much as is consumed day by day so much life is reborn as is consumed; as the flame of a candle is by the nourishment given by the liquor of the candle. He also l, "Where there is life there is heat, and where there is vital heat re is movement of the humors." Da Vinci propounded certain rules the investigator, among which are: "You must first have a theory, erwards practical work. There is no certainty where mathematics is involved; an experiment is a repetition of a natural process igned to discover the laws of relations presented by science; no ion of nature is without a cause. If you understand the cause you d no experiment; an experiment is never fallacious, only our erpretation of it may be wrong."

Sanctorious (1561-1636), Professor at Padua, made an avocation of ighing himself to determine the amount of "insensible perspiration" ring periods between the taking of food and when no sensible acuations had occurred. He estimated that there was half a pound s of moisture daily in the breath. His test for expired moisture was ade by breathing on a cold glass.

An understanding of nutrition was not possible until many dis-veries were made in the field of chemistry, since nutrition is a emical process. A list of the most important chemical discoveries of gnificance in paving the way to effective researches in nutrition is ven to provide a view of the landmarks along the road of progress nich have led to the present imposing monument of nutritional ience. Each discovery was a beacon which illuminated new ground d enabled the eyes of succeeding inquirers to discern new facts and lationships which hitherto had escaped detection.

In 1669 John Mayow heated antimony with a burning glass and owed that it increased in weight, and correctly interpreted the obser-tion to signify the formation of an oxide. He also showed that part the air was consumed in respiration. He placed a small animal and lighted candle in a closed jar, and noted that the candle went out st and the animal died later. If there was no lighted candle the imal lived longer.

1736. H. S. Duhamel De Monceau discovered sodium in plant h.

1743. A. S. Marggraf, the discoverer of sucrose, demonstrated the esence of phosphorus in plants.

1757. J. Black discovered carbon dioxide. This had been previously nown to Van Helmont, who had compared the properties of the gas olved in alcoholic fermentation and in the burning of charcoal, with at evolved when marble dissolved in acid, and the gas emanating om the Grotto del Cane, near Naples.

1761. A. S. Marggraf named potassium "Gewachslaugensalz" (Growth lye salt).

1774. J. Priestley discovered oxygen. His "fire air" disappeared during respiration and was replaced by "fixed air."

1770-1794. A. Lavoisier made clear, by experiments, the nature of respiration and of oxidation. He first used the thermometer and the balance in the study of metabolism. He believed that organic substances were composed of carbon, hydrogen, and oxygen.

1777. A. Crawford made experiments on animal heat and on combustion, and correctly interpreted his observations.

1779. A. J. Ingenhouss demonstrated the power of plants to utilize carbon dioxide, and their dependence upon chlorophyll.

1786. C. L. Berthollet showed that nitrogen was a constant constituent of animal tissues. Older chemists had found that ammonia was produced by dry distillation of both animal and plant tissues, but the role of nitrogen as a constituent of living matter gained recognition slowly.

1789. A. Fr. de Fourcroy distinguished three kinds of animal products on the basis of their nitrogen content and laid the foundation for the study of the proteins by noting the similarity of gluten of wheat and animal flesh, and by showing that plant juices afford a coagulum like animal protein when heated. He compared the coagulated proteins of plant juices with gelatin. He also discovered adipocere (corpse wax) and described cholesterol which he obtained from bile.

1742-1786. S. W. Scheele prepared and described tartaric, citric, oxalic, malic, lactic, uric, and gallic acids.

1789. J. H. Hassenfratz first demonstrated the presence of calcium, sulfur, and silicon in plants.

1789. C. A. Rückert combated the view that only organic substances in the soil were of value to plants. He held that the carbonic acid of soil dissolved inorganic nutrients, and emphasized the importance of soil analyses and the need of fertilizing soil with inorganic salts.

1798. L. N. Vanquelin discovered benzoic acid.

1804. Th. de Saussure, by numerous experiments, clarified somewhat the problem of the nutrition of plants.

1814. M. E. Chevreul discovered that fats are composed of fatty acids and glycerol. In 1832 he isolated creatine from muscle.

1828. Th. Wöhler synthesized urea from ammonium cyanate, thus demonstrating for the first time that an "organic" substance can be made "without the aid . . . of an animal."

1830. Liebig perfected methods for the quantitative determination of carbon, hydrogen, and nitrogen in organic compounds.

1839. Boussingault first undertook to make a balance of intake and

outgo of nutrients in food and excreta, using a cow as his subject. He published the first system of analysis of feeding-stuffs and fertilizers.

1849. Regnault and Reiset first devised and used a closed circuit system of apparatus for respiration experiments.

Early important discoveries were made concerning simple sugars and other carbohydrates, thus permitting the development of important concepts regarding the nutritive roles of these substances. In 1747 Marggraf discovered milk sugar, and in 1806 Prout discovered malt sugar. The latter also crystallized glucose from grape juice. Kirschoff, 1815, studied the acid hydrolysis of starch. Lagrange, 1817, discovered mannite, and observed that it was not fermentable. Several chemists established between 1830 and 1840 the empirical formula for glucose. Dubrunfaut, 1847, learned the true nature of malt sugar and fructose. The latter was formed through the acid hydrolysis of cane sugar. Hunefeld, 1836, discovered dulcit and Pelouze detected sorbose in 1852. Vogel discovered in 1815 the property of glucose solutions of reducing alkaline compounds of metallic salts. In 1870 von Beyer proposed the aldehyde nature of glucose. Tollens, Kiliani, and finally E. Fischer, greatly extended the knowledge of the structure of the different sugars after 1870. But not until 1875 was it discovered that glucose is the sugar of the blood. This important observation was made by Claude Bernard, only three years before his death.

Although casein of milk as it separates from sour milk, gelatin in cooled solutions in which meat had been boiled, egg white, and flesh of animals, were familiar examples of protein materials from time immemorial, no conception of their nature was gained until Beccaria, in the 18th century, described the gluten of wheat. Rouelle discussed the similarity of wheat gluten and the coagulated protein obtained from plant juices. Fourcroy, 1791, called attention to the similarity of plant and animal proteins. Bostock, 1808, first precipitated proteins with tannic acid and salts of heavy metals; and Prout, 1820, made the first elementary analysis of proteins. Bracconnot, 1822, first hydrolyzed proteins with acid. He discovered the amino acids glycine and leucine. Berzelius and Einhof, 1828, compared the analytical data from animal and vegetable proteins and found them to be alike. Mulder, 1838, coined the term protein. He believed that the sulfur and phosphorus content determined the differences in proteins, which, otherwise, he believed to consist of a nucleus of $C_{40}H_{62}N_{10}O_{12}$.

In 1872 Ritthausen wrote his book on the proteins of seeds, a notable contribution, describing as it did, a great amount of original work on the isolation of plant proteins. From this time onward the studies of Hoppe-Seyler, Weyl, Schmiedeberg, Drechsel, and others contributed many observations on the use of neutral salt solutions as solvents and, in stronger concentrations, as precipitants for proteins,

by means of which proteins could be purified without coagulating denaturing them. E. Fischer, Kühne, Hofmeister, Kossel, Osbo Chittenden, Van Slyke, Vickery, Dakin, Abderhalden, and a num of other workers, extended our knowledge of the properties of prote and their content of different amino acids which are formed on hydrolysis. Hoffmeister was the first to crystallize a protein. This accomplished by half saturation of egg albumen solution w ammonium sulfate.

Early Studies in Animal Nutrition.—Physiologists have alw welcomed the discoveries of chemists and physicists which they mig turn to account in their own experimental studies. As fast as n knowledge became available it pointed the way to new methods study. But even before any clear concept of the nature of food su stances was possible a few men sought to learn something of digesti and the processes of nutrition. Thus, contemporaneously with L voisier, Spallanzani (1729-1799), inspired by his experiments on re piration, demonstrated the consumption of oxygen and the expiratio of carbon dioxide by isolated glandular organs. He also studied dige tion by enclosing bits of foods in cages, which he inserted into th stomachs of birds, afterward recovering these by regurgitation or b withdrawal with a string. He swallowed such cages himself, and late drew them from his stomach. He found that foods which were no dissolved by water were rendered soluble by the gastric juice, an postulated chemical changes in digestion.

Magendie (1783-1855) first studied the unlike nutritive effects o the three outstanding types of food substances which are still accepte today, i.e., proteins, fats, and carbohydrates. He experimented wit dogs, restricting them to fatty, saccharine, and albuminous substances such as sugar alone, or butter or gelatin, together with distilled water and noted that they died in 30 to 36 days. He was the first to describ the eye condition known as xerophthalmia, now known to be caused b deprivation of vitamin A.

During the early decades of the nineteenth century the view o Hippocrates was generally accepted that there is but one kind o aliment, a universal nutrient substance present in various kinds o foods and which is abstracted from them during digestion. This view was reiterated by Richerand (1813) in his *Elements of Physiology* The outstanding early American investigator in the physiologica aspects of nutrition was William Beaumont, a surgeon in the U. S Army, who experimented during many years on his famous patient Alexis St. Martin. The latter, as the result of a gunshot wound, had a permanent external fistulous opening into his stomach. Beaumon published his classic *Experiments and Observations on the Gastri Juice, and The Physiology of Digestion* in 1833. He was satisfied wit

the idea that there is but one kind of aliment which, in digestion, is abstracted in the form of gastrite of aliment, the gastric juice furnishing the gastrite.

William Prout (1785-1850) published in 1834 his *Chemistry, Meteorology, and The Function of Digestion* (Lusk, '33). In this book he set forth the modern view that several kinds of nutrient substances are essential. These he distinguished as the saccharine group, the oleaginous group, and the albuminous group. He was familiar with the then known facts concerning the diversity of saccharine substances, among which were starch and several kinds of sugars; with a variety of oily and other fatty substances; and with gelatin and egg white and the solid protein material of cooked flesh after gelatin was dissolved out. He drew attention to fermentation of sugar into spirit of wine, or alcohol, as an example of the biological transformation of one kind of food into a totally dissimilar substance.

Gmelin, 1836, was the first to use the term Stoffwechsel (metabolism), a general term which includes all the chemical transformations which food undergoes in the constructive and destructive processes concerned with nutrition.

Regnault, 1849, first determined the respiratory quotient, or volume of carbon dioxide expired divided by the volume of oxygen consumed. With Reiset he studied oxygen consumption by animals of different sizes, and found that small animals used far more oxygen per unit of body weight than did large animals. It was ten times greater in sparrows than in chickens. They rightly assigned the difference to the greater relative body surface presented for cooling the creature of small weight. This was the beginning of the study of the relation of surface area to metabolic rate. This relationship was first clearly enunciated by Bergmann in 1845.

In 1852 Bidder and Schmidt published their book *Die Verdauungssäfte und der Stoffwechsel*. They described experiments which proved that energy metabolism, as represented by the amount of heat lost to the environment, is, in the fasting animal, a constant having the same value for animals with similar body volume, surface, and temperature.

Claude Bernard (1813-1878) studied the sugar of the portal and systemic blood and discovered that the liver could store glucose as glycogen, which he first prepared and described, and could reconvert it into glucose.

Mulder (1802-1880) was the first investigator to emphasize the protein element in nutrition. He examined protein materials from plant and animal sources, and stated his belief that protein is the most important of all known substances in the organic kingdom. This view received immediate recognition. His writings in 1838-1839 led Bouss-

ingault, in 1844, to assess the values of rations for farm animals largely on the basis of their content of nitrogen. Boussingault appreciated, however, that the non-nitrogenous principles were of value, and also that the mineral constituents in foods played an indispensable role.

The earliest attempts to analyze feeding-stuffs appears to have been made by Einhof about 1800. He compared the amounts of substances in different animal feeds which were soluble in water, alcohol, dilute acids, and dilute alkalies. The results were used by Thaer in Germany in 1809 (Armsby, '17) to express the values of different farm products in terms of "hay values." Good meadow grass was universally recognized to be a complete food, and it was naturally taken as a standard with which other feeds could be compared. This work was the beginning of experiments by animal husbandrymen in stock feeding, of a type which has persisted to the present day, and consists in comparing the results of substituting one feed for another in a ration. After decades of trials of many kinds to determine the values of foods by chemical analysis, a procedure was evolved by Professor Henneberg, which was known as the Weende method, and which was presented at the second Convention of German Agricultural Chemists in 1864. By this method the protein, carbohydrate, fat, mineral matter, and moisture were estimated.

Liebig exerted great influence on the thoughts of physiologists and of practical feeders of animals. In his famous book on *Animal Chemistry,* published in 1846, he stressed the idea that only those nutrients which could be transformed into blood were of value to the organism. He stated that the investigation of substances adapted to this purpose should be restricted to the determination of the composition of foods and comparison made thereof with the make-up of blood.

Liebig believed that such physiological functions as muscular contraction and glandular activity appeared to proceed at the expense of the albuminous tissue structures. The function of nutrition, therefore, was to replace destroyed tissue protein. The nitrogenous group of nutrients he designated plastic foods. Fats, starch, sugars, beer, wine, brandy, he called respiratory foods, which served as a source of heat. Liebig's view that nutrition was identical with reconstruction of protein in the tissues which had been destroyed by work persisted for many years, due largely to the force of his great reputation.

At the beginning of the last third of the nineteenth century, the Munich School of physiologists, under the leadership of Carl Voit, was the center of learning and progress in the field of nutrition. Voit showed that geese could transform carbohydrate into fat. Workers influenced by his school demonstrated that the muscles could do work at the expense of carbohydrate or fat, and that a part of the protein in the food could be converted into carbohydrate. It is not possible

ere to follow the progress of such experiments further than to point
ıt that Voit's school demonstrated the dual function of carbohydrate
ıd protein and the interchangeability, to a certain extent, of these
ıbstances.

Voit established the fact that even when the daily protein intake
high, protein is not stored, but is, except in the growing animal,
egraded during a period of about 24 hours into urea, and other
etabolic end-products of protein metabolism, and these products are
creted in the urine. Fats may be retained in large amounts and
eposited in the adipose tissues. Carbohydrates may likewise be stored
glycogen in liver and muscle, after being digested and transformed
to glucose. When a need arises glycogen stores may be reconverted
to glucose, which is contributed to the blood. If carbohydrate is
gested in excess over the requirements for energy (which may also
derived from fats or proteins) it may be converted into fat and
eposited as an energy reserve.

With the perfection, through the years, of chemical methods for
e analysis of foodstuffs, it appeared to students of nutrition that
ıtural foods consist, aside from cellulose, lignin, pentosans, waxes,
c., of protein, carbohydrates (starch, sugars), fats, and ash constitu-
ıts. Since it was found that isolated proteins contained, on an average,
ıout 16 per cent of nitrogen, and since direct estimation of proteins
as not possible, it became the custom of chemists to estimate the
otein content of foods by multiplying the content of nitrogen by the
ctor 6.25. Generally the carbohydrate fraction was determined by
fferentiation between cellulose and "nitrogen-free extract." The latter
ntained the pentose sugars, which are present in notable amounts
many vegetable foods, and are prominent constituents of straw, as
ntosans. Although it was well recognized that the methods of food
ıalysis were only approximate, the results of separate determinations
the above-named components, summed nearly enough to 100 per
nt to convince investigators that the chemist was actually estimating
l of the important constituents of foods. Hence the conclusion that
oteins, fats, carbohydrates, and certain mineral elements, were the
ıly nutrients which man and animals require.

With the accumulation of experimental data from animal feeding
ıdies, it became evident that the quality of a ration could not be
curately predicted on the basis of its chemical composition. There-
re, animal husbandrymen began to speak of "the specific effects of
ıtrients" to account for the better results obtained from certain
tions when one food was included, as compared with the same
ixture with one constituent substituted by another. It was not until
ıout 1900 that chemical studies showed that proteins from different
urces yielded on digestion very different proportions of the then

known amino acids. Other amino acids were discovered from time t time, and by 1900 about 16 were known. It was easy to account f observed differences in the quality of foods by assuming that the cau lay in the quality of their proteins. This was a correct judgment, b as later studies showed, was by no means the only factor involved.

Atwater (1895), a student of Voit, returned to America about 187 (Chittenden, '30) with a glorious program of research. He believe that if he knew the chemical composition and the fuel values of a important foods and feeding-stuffs, in terms of their content of protei carbohydrate, and fats, and the digestibility factors for these, togethe with the energy requirements of human beings and animals, it woul be possible to place nutrition of man and animals on a sound eco nomic basis. He set to work, with the support of the U. S. Departmen of Agriculture, to analyze all American foods, taking into accoun values as purchased, and waste in preparation. In 1895 he published a compilation of food analyses, emphasizing their energy-yielding func tions, and discussed both the chemistry and economy of foods. To illustrate wise and unwise food purchases he presented a table showing the amounts of nutrients which could be purchased in the Eastern United States in 1895 for 25 cents. The following items are selected to illustrate his advice.

25 cents will pay for:

	Total Pounds	Protein Pounds	Fat Pounds	Carbohydrates Pounds	Calories of Potential Energy
Milk @ 8 cts. per qt. . . .	.81	.23	.25	.29	2,020
Cheese @ 18 cts. per lb. . .	1.32	.96	.40	.49	2,850
Potatoes @ $1.00 per bu. . .	2.69	.27	.01	2.29	4,785
Sugar @ 5 cts. per lb. . . .	4.90	0	0	4.89	9,095
Dried beans @ 5 cts. per lb. .	4.37	1.15	.10	2.96	8,065
Corn meal @ 3 cts. per lb. . .	7.08	.77	.32	5.88	13,720
Wheat flour @ 3.5 cts. per lb.	6.25	.79	.07	4.68	10,285
Oatmeal @ 5 cts. per lb. . .	4.62	.76	.36	3.41	9,275
Eggs @ 35 cts. per doz. . .	.23	.12	.10	0	645

Atwater regarded fruits and water-rich fresh vegetables, eggs, etc., as extravagent food purchases. He saw no reason why one should not remain in health while taking a diet selected from the cheapest dried food products.

Atwater visualized the coming of a time when farmers should be able to consult tables showing the cost of protein and energy in various farm crops, and taking into account digestibility of their food ele-

nents, to select the cheapest sources of these nutrients for compounding their rations for feeding animals. Fortunately for his peace of mind he never saw the effects of restricting animals or men to diets which might have been compounded on his advice. It is also very fortunate that housewives did not, so far as we are aware, attempt to follow his advice in the feeding of their families.

Voit set the average protein requirement of a man at 118 gm. per day. The value was placed at 145 grams for men doing hard work. Atwater thought 125 grams the appropriate figure for men doing an ordinary amount of work. For several decades these standards were followed in estimating the nutritive requirements of soldiers, prisoners, and laboring men where group feeding was practised. The stimulus given by Voit and by Atwater to nutrition studies resulted in many dietary studies in different parts of the world. Dietary studies were made on laborers, sedentary workers, students, Alabama negroes, Mexican families, football teams, and others. McCay studied the dietaries of various peoples in India, where the protein consumption varied from 67 grams daily for Bengali students, to Bhutias taking 150 to 160 grams daily. He stated (McCay, '12), "We may conclude this study of the effects of the level of protein metabolism on the physique and general efficiency of different tropical tribes and races by stating that the facts afford ample proof of the all-important influence exerted by food, and particularly protein, in determining the degree of muscular development, the general physical endowment, the powers of endurance, resistance to disease, and most important of all, the place a tribe or race has won for itself in manliness, courage, and soldierly instincts . . . the higher the level of protein interchange, the more robust and energetic, and the more manly the race."

There were, during half the century following 1875, many differences of opinion expressed concerning man's requirement for protein. While C. Voit and Atwater laid emphasis on the point that it was hazardous to transgress below the limits they set for protein consumption, Hirschfeld remained in nitrogen equilibrium for a fortnight on 30 grams of protein intake daily, and E. Voit found vegetarians to be in nitrogen balance on 50 grams daily of protein, augmented by fats and carbohydrates to meet their energy requirements. Several other investigators confirmed the fact that protein equilibrium was attainable at these low planes of intake. C. Voit, himself, enunciated the principle that the smallest amount of protein, in addition to energy sources as fats and carbohydrates, capable of maintaining the body in health and vigor is best, but he did not amplify his view as to this requirement.

In 1901 Professor Russell Chittenden, at Yale University, turned his attention to a study of the protein requirements of man. Horace Fletcher and Dr. Van Someren, a vegetarian, had found each other

excellent company because of their common interest in the alleg virtues of intensive mastication. They had convinced themselves th careful attention to the mastication of each morsel of food, as long it could be kept under control in the mouth, made the appetite mo discriminating, diminished the total requirement of food by one-ha and reduced the protein requirement by two-thirds! They submitte themselves to the observation of Sir Michael Foster, and had th gratification of demonstrating these convictions by experimenta methods. In 1902-03 Fletcher spent several months with Dr. Chit tenden, and demonstrated that he was able to maintain his weight a 75 kilos and perform the work of a trained athlete in the gymnasiun on about 43 grams of protein daily.

Chittenden instituted prolonged experiments on 5 brain worker (professors), 13 soldiers, and 8 university athletes, who worked hard both mentally and physically. During 225 days a careful record wa kept of food consumed, and the excreta were analyzed chemically. Dr. Chittenden, himself, who weighed 57 kilos, maintained himself in nitrogen equilibrium and greatly improved his health, on 36 grams of protein daily, with a calorie intake of 2000. Dr. Mendel, who weighed 70 kilos, was in equilibrium on 41 grams of protein. The soldiers and athletes were in equilibrium on 50 and 55 grams of protein respectively. All attested the maintenance of perfect health on these low protein levels. Chittenden crystallized his view that the low protein mixed diet is most conducive to health for the following reasons:

Because protein cannot be stored up in the tissues.

Because energy is dissipated in excreting the surplus protein.

Because excess of protein tends to disseminate toxins throughout the body, arising through putrefaction in the colon.

Because there is no necessity to engage in muscular work to aid in working off excess of protein, hence energy is saved.

Because a combination of animal and vegetable proteins is essential to satisfy the requirement of the body.

The low protein regimen advocated by Chittenden was characterized as *physiological economy in nutrition.* His books (Chittenden, '04, '07) *Physiological Economy in Nutrition* and *Nutrition of Man* were published in 1904 and 1907 respectively. In 1909, Sir James Crichton-Browne ('09) published his *Parcimony in Nutrition,* in which he controverted Chittenden's views. He asserted that "the success of the races, their vitality and energy, might almost be measured by the degree in which animal flesh has entered into their diet. All the successful races have habitually consumed protein far in excess of the Chittenden standard and far in excess of what was required for

ssue repair, and when we find a definite relation between protein onsumption and racial success there is good ground for believing that ehind it there is a biological law."

The controversy over the role of the protein element in nutrition as the most outstanding feature of discussions relating to diet during ne first decade of the twentieth century. The observations underlying nis discussion were not of a nature to lead to progress in the under-tanding of the fundamentals of nutrition. Meanwhile animal experi-nenters were puzzling their heads over the cause or causes which etermined the extraordinary differences in the nutritive values of ations which on chemical analysis appeared to be of similar composi-on. Feeding experiments conducted with mixtures of natural feeding-uffs, and the by-products of the milling industry and the slaughter ouse, were not of a nature which could lead to an understanding of ne problem. A new method of approach to the study of the nutritive equirements of the body and the properties of individual foodstuffs as necessary. Unknown to those who were vocal in that decade in xpressing their views about diet there lay buried in the literature of hysiology and biochemistry, several fundamentally important con-ibutions which pointed the way to an experimental procedure which as destined to eventually clarify the problems in this department of nowledge and to create a science of nutrition as a department of the cience of chemistry. In the following chapter the early development f the modern method of study of nutrition problems which resulted n a clear statement of the nutritive requirements of the body, and the oncept of dietary essentials, will be briefly reviewed.

REFERENCES

thenaeus. The Loeb Classical Library, G. P. Putnam's Sons, New York.
HIEF STANDING BEAR (1933). "The Land of the Spotted Eagle." Houghton Mifflin Co., New York.
arach (1928). Ann. Med. History, *10,* 387. (Discusses Diodorus.)
lippocrates. The Loeb Classical Library, G. P. Putnam's Sons, New York.
ARRISON (1929). "An Introduction to the History of Medicine," 4th ed., W. B. Saunders Co., Philadelphia. (Discusses Erasistratus.)
ALSH (1927). Ann. Med. History, *9,* 132. (Discusses Galen.)
ERZFELD (1926). "Leonardo da Vinci, der Denker, Forscher und Poet, aus seiner veröffentlichten Schriften." E. Diederichs, Jena.
ICHERAND (1813). "Elements of Physiology." T. Dobson, Philadelphia.
EAUMONT (1833). "Experiments and Observations on the Gastric Juice, and the Physiology of Digestion." F. P. Allen, Plattsburgh.
USK (1933). Clio Medica X, "Nutrition." Paul B. Hoeber, Inc., New York. (Discusses the contributions of Prout, Gmelin, Regnault, Bidder and Schmidt, Claude Bernard, Mulder, Liebig and Voit.)

ARMSBY (1917). "The Nutrition of Farm Animals." The Macmillan C New York.

ATWATER (1895). "Chemistry and Economy of Food." U. S. Dept. Agriculture, Bull. No. 21.

CHITTENDEN (1930). "The Development of Physiological Chemistry in t United States." The Chemical Catalog Co., New York.

MCCAY (1912). "The Protein Element in Nutrition." Edward Arnol London.

CHITTENDEN (1904). "Physiological Economy in Nutrition, with Speci Reference to the Minimal Proteid Requirement of the Healthy Ma an Experimental Study." F. A. Stokes Co., New York.

CHITTENDEN (1907). "The Nutrition of Man." F. A. Stokes Co., Ne York.

CRICHTON-BROWNE (1909). "Delusions in Diet; or, Parcimony in Nutr tion." Funk and Wagnalls Co., New York.

CZAPEK (1905). "Biochemie der Pflanzen." G. Fischer, Jena. (Gives refe ences to discoveries in the chemistry of animal and plant products.)

CHAPTER

II

Nutritive Requirements of the Body, the Modern Concept of Dietary Essentials

T IS to the merit of Magendie (1816) that during the first quarter f the nineteenth century he introduced the experimental procedure of eeding to animals diets which in composition were simplified to a egree that made it possible to name their chemical constituents. His nowledge of what constituted purity in food substances such as roteins, fats, and carbohydrates was necessarily very limited since hemical science was in its infancy. Certain notable investigators never ost sight of his demonstration that animals could not remain in ealth when fed certain simple mixtures containing the "staminal rinciples," the saccharine, oleaginous, and albuminous. Pereira (1843), n his *Treatise on Food and Diet,* discussed the views of Prout, saying hat after mature consideration he was satisfied of the impossibility of educing all nutritive principles to these four heads. Common salt, for xample, could not be considered only as a luxury, but as a substance s essential to life as nitrogenous or non-nitrogenous food and water. He pointed out that lemon juice, which is now recognized as one of ur most valuable antiscorbutic foods, does not owe its nutritive mportance to water, sugar, albumen, or oil.

Voit (1881, p. 19) clearly shared this view when he wrote "Unquestionably it would be best for the purpose (experimental study) f one could feed only pure chemical compounds (the pure foodstuffs), or example, pure protein, fat, sugar, starch, and ash constituents, or nixtures of the same. However, inasmuch as men and also animals nly rarely tolerate continuously such tasteless mixtures, it is necessary n most cases to choose foods as they are provided by nature. Neverhcless, it would probably be possible and very desirable to repeat the ests with the natural food products by using the pure substances, lthough the results yielded thereby might not be essentially different."

Voit's words do not intimate a suspicion that the predicted favorable outcome of such experiments might be attributable to a cause other than loss or perversion of appetite. The first to put su experiments to test was Lunin (1881) whose experiments were p formed in the same year. He employed a diet of casein, milk fat, a cane sugar, to which, in different experiments, he added varic inorganic salts including an imitation of the ash constituents of mi Mice survived but a short time on the ash-free diet and about doul the time with certain mineral additions. He found it impossible keep mice in health on his diets. His illustrious teacher, G. v. Bung raised the question whether milk did not contain nutrients of u known nature which were indispensable for health.

Pekelharing ('05), in Holland, restricted mice to a diet consistin of bread baked with casein, albumin, rice flour, lard, and a mixture c all the salts which he thought ought to be in their food. When the were given this ration with water the mice eagerly nibbled the foo for a few days, but they soon began to lose their appetite, grew thin and in 4 weeks all were dead. He observed that when they had mil to drink, in addition to this bread, they remained in good healt although the quantity of albumin, lactose, and fat which they as similated from the milk was "quite negligible in comparison with what the bread on which they fed contains." He stated that the element in the milk which kept the animals alive also occurred in the whey from which casein and fat had been removed. He wrote "My intention is to point out that there is a still unknown substance in milk, which, even in very small quantities, is of paramount importance to nourishment. If this substance is absent, the organism loses the power properly to assimilate the well-known principal parts of food, the appetite is lost and with apparent abundance the animals die of want. Undoubtedly this substance not only occurs in milk but in all sorts of foodstuffs, both of vegetable and animal origin."

Hopkins ('06) wrote "But further, no animal can live upon a mixture of pure protein, fat and carbohydrate, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or other animals, and these contain countless substances other than the proteins, carbohydrates and fats. Physiological evolution, I believe, has made some of these well nigh as essential as are the basal constituents of the diet; lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something familiar, and a substance which happens to have been tried. The field is almost unexplored, only it is certain that there are many minor factors in all diets, of which the body takes account. In diseases such as rickets, and particularly in scurvy, we have

ad for long years knowledge of a dietetic factor, but though we now how to benefit these conditions empirically, the real errors in he diet are to this day quite obscure. They are, however, certainly f the kind which comprises these minimal qualitative factors that I m considering. Scurvy and rickets are conditions so severe that they orce themselves upon our attention, but many other nutritive errors ffect the health of individuals to a degree most important to themelves, and some of them depend upon unsuspected dietetic factors."

Hopkins reported that when rats were fed a food mixture conining casein, starch, cane sugar, lard, and inorganic salts, they rehained alive and grew a little when the components were supplied n a crude condition. When they were carefully purified, growth nvariably ceased in a very short time and the animals declined and ied. He showed that their failure was not due to insufficient food ngestion. When a small daily allowance of whole milk was given, which did not provide more than 4 per cent of the food eaten, the ats were able to grow to a certain extent. These experiments marked he beginning of an appreciation of what Hopkins called the "accessory actors of the diet."

Takaki (1887) reported a remarkable investigation on the prevenion of beriberi by diet. About the year 1880 this disease had become scourge among Japanese sailors. Takaki became impressed with he superior health of sailors in the British Navy, as compared with hat of men in the Japanese Navy, and set about securing an improvement of the diet of the latter, which he judged to be of inferior quality. After many vicissitudes caused by resistence of officials, he succeeded n increasing the content of meat and vegetables, and of introducing ondensed milk into the rations of the sailors. Through his influence training ship was commissioned to make a nine months cruise, the nen receiving the standard ration. While during this cruise there were o cases of beriberi among the officers who had a superior diet, there ccurred 169 cases of the disease among 276 sailors. Another ship was nen commissioned to make the same cruise, using the improved ietary. There were but 14 cases of beriberi among 276 men on this ruise. The results were so striking that a reform in the rationing of ailors was instituted which resulted in practical eradication of the isease from the Navy.

Hopkins was apparently not aware, in 1906, of the studies of Takaki, nor those of Eijkman (1897) in Batavia, who had produced xperimentally in fowls a disease similar to beriberi, which had for enturies constituted one of the major causes of ill health among ice-eating peoples. Eijkman found that chickens restricted to polished ce developed polyneuritis, the cardinal symptom of beriberi, whereas ney did not do so when fed unpolished rice. He further demonstrated

that a water or an alcoholic extract of rice polishings would proc dramatic cures of polyneuritic birds.

Eijkman did not at first correctly interpret his experimental ob vations. He concluded that rice, over-rich in carbohydrate, produ in the intestine a substance which acted as a poison to nerve cells, that the outer layers of the seed contained a pharmacological antid Grijns ('01) expressed the view that beriberi, in man and in birds the result of a deficiency in an essential nutrient. Eijkman's interp tation of his findings furnishes an illustration of the difficulty wh pathologists had in accepting the view that a deficiency of anythi could cause disease. This was recognized in 1919, in the *Report on t Present State of Knowledge Concerning Accessory Food Factors* (*Vi mines*), prepared for the British Medical Research Committee ('19

"Disease is so generally associated with positive agents—the par site, the toxin, the *materies morbi*—that the thought of the patholog turns naturally to such positive associations and seems to believe wit difficulty in causation prefixed by a *minus* sign. Even in connectio with deficiencies arising within the body there is or was a simila tendency. When the importance of the internal secretions was firs recognized there seemed to be much hesitation in believing tha symptoms might be frankly due to their failure. When each fresh internal secretion was described there was always an effort to show that its function was to 'neutralize' some, always hypothetical, toxic substance. Symptoms, on this view, were due to the unmasking of a deleterious agent rather than to simple deficiency in a normal and necessary agent. To distinguish between these two possibilities was of course a scientific duty; but, at any rate in the earlier literature of internal secretions, a bias against the simpler view interfered with the fair interpretation of experimental results. So (A similar situation existed) in connection with the newer conception of disease as due to dietetic deficiencies."

Stepp ('09-'12) carried out significant experiments to determine whether lipids are essential for normal nutrition. He found that mice when fed wheat bread and milk, remained in health during several months, but the same foods, after thorough extraction with alcohol and ether, did not support life longer than one month. Restoration of the extract made the food complete once more. He tried adding cholesterol, lecithin, kephalin, or cerebrone, but found that these were not able to protect mice against early nutritive failure. Stepp wrote "It is not impossible that unknown substances indispensable for life go into solution with the lipids, and that the latter thereby become what may be termed carriers for these substances."

Impressed by the investigations of beriberi—notably those of Fletcher, Fraser, and Stanton, Schaumann, Grijns, and others—Funk

began at the Lister Institute in London, in 1911, an attempt to isolate the antiberiberi substance from rice polish. Similar attempts, but less comprehensive, were made by others. He succeeded in making a far more potent concentrate of the curative substance than had been hitherto obtained, but did not succeed in isolating it. In 1912 Funk ('12-13) published a paper entitled "The Etiology of the Deficiency Disease," in which he introduced the term vitamine. This paper attracted a great deal of attention and stimulated much thought by others. After reviewing the literature relating to scurvy, beriberi, and pellagra, he expressed the opinion which was to be later verified, that these were all to be considered deficiency diseases. He suggested further that rickets and sprue were in the same category.

Vitamin A.—Beginning in the year 1907 McCollum and his associates, (McCollum and Davis, '13) at the University of Wisconsin, initiated studies with diets of purified foodstuffs. These studies were stimulated by the observations of Lunin and of Hopkins. McCollum and coworkers had not learned of the writings of Pekelharing, already referred to, since they appeared in Dutch and were not generally known. In 1909 Osborne and Mendel ('11, '13-14) of Yale University, undertook similar investigations. After many vicissitudes and wrong interpretations, due to the fact that certain ingredients of their mixtures, notably milk sugar, were not sufficiently purified, and carried significant amounts of "accessory food substances," these two groups of workers were able to demonstrate that certain fats, but not all, carried an indispensable nutrient. Thus McCollum and Davis found that a certain diet, composed of purified foodstuffs, proved a failure in the nutrition of young rats when it contained lard as the fat constituent, but was far more satisfactory when butter fat or ether extract of egg yolk was substituted for the lard. They, thereupon, announced the discovery of a hitherto unknown fat-soluble nutrient, which had been foreshadowed by the studies of Stepp. Later this factor was named vitamin A.

In 1921 Steenbock and his coworkers showed that carotene, the yellow pigment of carrots and of many other yellow and green vegetable foods, when fed to rats had a vitamin A-like effect. This conclusion was controverted by several investigators, but in 1928 von Euler and his coworkers reopened the question. They pointed out that at the time of Steenbock's investigation vitamin D was unknown and that in its absence young rats could not develop normally; hence a clear demonstration of the effects of vitamin A on health and growth could not well be made. They administered vitamin D together with crystalline carotene, and confirmed the findings of Steenbock that the yellow pigment could serve as a source of vitamin A. It was clear, however, that vitamin A itself was not a yellow pigment

since almost colorless fish liver oil contained it in abundance. Vitar A must, therefore, be formed in the body from carotene, a surm which was later fully justified by experiments. Vitamin A is n known to occur preformed in various liver oils and oils from differ mammals. It does not occur in vegetable substances, so far as is knov but is found there in the form of its precursors, carotene, cryptoxanthin.

Osborne and Mendel, after failing to secure growth or survival young rats on a diet of purified casein, starch, lard, and a s mixture, had the happy thought to prepare an evaporated whey, fro which both casein and lactalbumin had been removed, as an adjuva to their purified food mixture. When 27 per cent of the diet consiste of this "protein-free milk," they succeeded in growing and maintai ing rats for a year or more in apparent health. They regarded the "protein-free milk" as essentially a source of a well-balanced sa mixture, but later, in the light of accumulating knowledge, they cam to realise that it supplied other essential nutrients. From this produc they proceeded to make up an "artificial protein-free milk" fron "carefully purified lactose" and inorganic salts, and discovered that thi mixture was very inadequate as compared with the "natural" product At one time they reported (1912) that all true fats were dispensable in the diet, and that their experiments pointed in the same direction with regard to the lipids in general. But the following year (1913) they were led to announce as the result of early failure of rats on their "artificial protein-free milk" made of more highly purified materials, that the addition of butter (containing about 15 per cent of water, curd, salts, and whey constituents) caused an extraordinary improvement in the growth and general condition of their animals. On November 4, 1913, they reported similar recoveries of their animals when butter fat was employed instead of butter, and differentiated clearly between the growth-promoting properties of butter fat and of cod liver oil, in contrast to lard and almond oil, as had McCollum and Davis between butter fat and egg yolk fats on the one hand, and lard and olive oil on the other. McCollum and Davis had saponified butter fat and shaken the resulting soaps with olive oil thus transferring the growth essential to the olive oil.

Proteins.—Osborne and Mendel, using their "protein-free milk" as the source of unidentified dietary essentials, proceeded to study the dietary values of different purified proteins. Steady progress had been made in the discovery of new amino acids formed by the hydrolysis of proteins. A method was devised by Hausmann for differentiating quantitatively yields of ammonia and mono- and di-amino acids. By means of this method it was shown that proteins were very differently constituted as respects their yields of these constituents. E. Fischer

('06) in 1902 developed a much more elaborate method of analysing proteins for their content of individual amino acids. The method was applied between 1904 and 1909 by Osborne to the examination of many purified proteins. These studies clearly indicated that proteins should have very different nutritive values, but nothing was known about the significance of individual amino acids in nutrition. It was this field which was next explored by Osborne and Mendel. They brought to light the fact that one of the diamino acids, lysine, is the limiting factor in determining the growth value of a number of vegetable proteins. In others the first or second limiting factor was tryptophane, and in still others the sulfur-containing amino acid, cystine was regarded as the limiting factor. Their cystine was probably contaminated with the essential sulfur-containing amino acid methionine, then unknown. They demonstrated by their experiments the importance of the source and nature of protein in the diet, rather than its quantity, as a factor in nutrition. The field is still being explored to determine which of the 23 now known amino acids derived from the digestion of proteins are dispensable and which are indispensable in mammalian and avian nutrition.

Deficiencies of Individual Natural Foods.—McCollum and Davis ('15) turned their attention to determining the chemical nature of the limiting nutritive factors in individual natural foods. They found that wheat, maize, or oat grains were effectively supplemented for the production of growth in young rats by the addition of calcium, a purified protein (casein), and butter fat, which showed that these grains were deficient in calcium, in certain essential amino acids, and in the newly discovered nutrient in certain fats. The oat kernel, however, was less well supplemented by these additions than were wheat or maize. The cause remained unknown until Smith and Hendrick, in 1926, demonstrated that a heat-stable vitamin, soluble in water, which was hitherto unsuspected, is supplied by oats in smaller amount than in the other grains mentioned.

When they applied the same supplements to polished rice they found that the young rats immediately suffered nutritive disaster. The studies of Eijkman (l.c.) afforded a clue to the cause of these failures. When, in addition to calcium, casein, and butter fat, an alcoholic extract of wheat germ was added to polished rice, growth was secured. They were led by these results to assert that in addition to protein, carbohydrate, fats, and the essential inorganic salts, two unidentified nutrients "fat-soluble A" and "water-soluble B" were essential for growth and the maintenance of health.

The Antiscorbutic Vitamin.—Scurvy was for several centuries rightly regarded as a disease due to dietetic error. Apparently the first people to learn how scurvy can be cured were the Indians of Canada

(Biggar, '24), who must have been long familiar with the disease winter. An Indian advised Jaques Cartier, who lost 26 of his p from scurvy while wintering on the St. Lawrence River in 1536 give his sick men an infusion of spruce needles, a remedy which immediately successful in restoring them to health. Bachstrom 1734, wrote on scurvy and attributed the disease to lack of "fr fruits of the earth, and greens." James Lind (1753) experimenting scorbutic sailors, clearly demonstrated the virtues of oranges a lemons in curing the disease. Yet failure of lime juice to prev scurvy in the crews of the *Alert* and the *Discovery,* commanded Sir George Nares on an expedition to the Polar Regions in 1875-18 and in the British Army Garrison at Chitral, India, in 1895-1896, a in the party of Captain Scott while on their voyage to the Antarc in 1902, tended to unsettle the view that scurvy was due to dieta deficiency (Medical Research Council, '32). There can be no dou that in the above-mentioned cases the lime juice was inferior, perha adulterated, or heated to preserve it, thus destroying its antiscorbu property.

The demonstration of a nutritive deficiency in scurvy was provid by Holst and Fröhlich ('07, '12), who showed that scurvy could induced in guinea pigs by restricting them to a diet of grains an water. Supplementing the diet with fresh vegetables, fruits, or fru juices resulted in the maintenance of health in the animals. Heatin the antiscorbutic foods reduced or destroyed completely their pr tective value. The expressed juices of fruits or vegetables, they foun rapidly deteriorated in their antiscorbutic value on keeping, bu citrous fruit juices and sorrel juice were more stable in this respec It was evident that a specific antiscorbutic substance existed, an biochemists turned their attention to its isolation and identific tion.

Szent-Györgyi ('28), in a study of the nature of substances involve in the oxidation-reduction system in adrenal cortex, orange, and ca bage, isolated a specific substance and identified it as a hexuron acid. Waugh and King ('32) isolated the antiscorbutic vitamin an identified it with Szent-Györgyi's compound. In the same year Svirbe and Szent-Györgyi ('32), confirmed the antiscorbutic value of th substance, which on further investigation proved to be not an uron acid but a furane carboxylic acid. Its synthesis followed immediate (Hirst and coworkers). Tillmans and associates ('33) introduced th indicator 2:6-dichloro (or dibromo-) phenolindophenol as a titrant fo the estimation of the vitamin, which was given the name ascorb acid. The way was laid open for precise studies of the many inte esting problems in the distribution, conditions of stability, and phys ological requirements of the antiscorbutic substance.

The Antiricketic Vitamin.—Although cod liver oil had been cognized for a century or more as a specific remedy for the prention or cure of rickets, the etiology of this disease remained a ystery. New ground was broken by E. Mellanby ('18, '19, '20), who duced experimental rickets in pups by feeding them diets consisting bread or cereal, milk, linseed oil, yeast, and salt with or without ange juice. Ricketic pups were given various kinds of fats and from e response it was clearly evident that certain of these exerted narkable effects in protecting the animals against rickets. Cod liver was far superior to all other fats tested. He was inclined to entify the antiricketic factor with vitamin A, but stated that the tiricketic value of certain vegetable fats, e.g. peanut oil, was greater an could be accounted for on the basis of its vitamin A content. ellanby established the fact that there exists an antiricketic substance. McCollum, Simmonds, Parsons, Shipley and Park ('21) undertook 1918 an elaborate study of the characteristics of the diet which ould cause the development of acute rickets. In 1920 they published description of 5 diets, one of which contained cod liver oil, and scribed the histological appearance of the bones of young rats stricted to these regimens. They, as well as Sherman and Pappenimer ('21), emphasized the importance of the ratios between calcium d phosphorus in the experimental production of rickets. The Baltiore group also emphasized the role of certain fats in ricketsevention. More than 300 diet formulae were employed and histoical studies were made on the bones of about 2500 rats. It was ncluded that diets deficient in calcium but rich in phosphate, in the sence of certain fats or of sunlight, caused the rapid development rickets complicated with tetany. Diets deficient in phosphate but h in calcium, in the absence of certain fats or sunlight, caused kets uncomplicated with tetany. In the prevention of rickets cod er oil was the most potent of the fats tested. Butter fat, when fed the extent of 20 per cent of the diet, exerted some protection as a sample of coconut oil. The coconut oil was devoid of vitamin A ce rats given this fat, in an otherwise A-free diet, developed ophthalmia. Hopkins had shown that blowing air through heated ter fat destroyed vitamin A. The Baltimore Group of investigators cCollum et al., '22) destroyed the vitamin A of cod liver oil by s means and observed that its antiricketic value was still retained, ts demonstrating that a second fat-soluble vitamin existed, which y named vitamin D. Rats kept on rickets-producing diets, they erved, did not develop rickets when exposed daily to sunlight, a firmation of the observation of Huldschinsky ('19). Schmorl ('09) described in detail the reformation of the provisional zone of ification on the epiphyseal side of the metaphysis, which is the

first sign of healing of rickets in children. On the basis of this ch
McCollum, Simmonds, Shipley and Park ('22) described a de
biological test for calcium-depositing substances, the so-called
test" which still remains the most satisfactory method for assay o
antiricketic substances.

Relation of Vitamin D to Ultraviolet Rays.—The season
cidence of rickets and its geographical distribution had for
supported a vague opinion that rickets was in some way assoc
with sunshine, fresh air, and other hygienic factors in the c
environment. Huldschinsky (l.c.), working with German chil
during and after the World War, was the first to establish the
that ultraviolet irradiation constituted a specific cure for rickets. F
time there appeared to be no way of reconciling the apparent
tradiction of the existence of a specific antiricketic dietary facto
well as a specific antiricketic action of ultraviolet rays. The prob
was solved simultaneously and independently by Hess and Weins
('24) of New York, and Steenbock and Black ('24) of Wisco
who announced in 1924 that antiricketic potency could be develo
in various foodstuffs by exposure to the rays of a mercury va
quartz or carbon arc lamp. Using the "line test," and the conten
ash in the bones as criteria, it was soon demonstrated that puri
protein, carbohydrates, fats, and mineral elements do not acq
antiricketic potency on irradiation. Cholesterol, and other sterols fr
plants, did show this property. Hess and Windaus ('26-27) repo
that if cholesterol is purified by the formation of the dibromide,
the cholesterol is regenerated, the purified substance cannot
activated by irradiation. This pointed to an impurity in cholest
as the provitamin D. Rosenheim and Webster ('26) compared
absorption spectrum of cholesterol purified by repeated crystall
tions, with that which had been purified through the dibrom
and observed that certain lines were absent in the spectrum of
latter. They announced that the "impurity" in ordinary choleste
after many crystallizations, was ergosterol, a sterol abundant in oi
ergot. This sterol had been isolated and studied by Tanret. Ergoste
after irradiation, protects rats against rickets when administered
doses equivalent to 0.0001 milligram daily. The presence o
provitamin D in the skin accounts for the cure of rickets by sunli

The B-Complex.—It was assumed for years that extracts contain
"water-soluble B," later called vitamin B, owed their dietary
nificance to a single substance, the antineuritic factor. Smith
Hendrick ('26) repeated the experiment of McCollum and Davi
supplementing rolled oats with casein, butter fat, and a calcium
The latter workers had exhibited growth curves which were far be
normal, showing that the supplementation by these additions was

omplete. Smith and Hendrick demonstrated that the addition of east, or yeast extracts containing very little protein, exerted a emarkable improvement in growth of rats on the oat diet. Since east or yeast extracts which had been heated sufficiently to destroy he antineuritic vitamin were as effective in improving the condition f the animals as the unheated material, it was evident that the mprovement of the diet was not due to supplementation with any ecognized nutrient, but to a heat-stable factor distinct from the ntineuritic substance. Goldberger (Goldberger and Wheeler '27) was ed to believe, by his studies on human pellagrins and on dogs uffering from "blacktongue," a condition supposed to be the analogue f pellagra in man, that the heat-stable water-soluble vitamin of mith and Hendrick was the antipellagra vitamin. There is still eason to believe that his surmise was correct, although it is now nown that there are contained in such extracts, as were then used, everal indispensable nutrients, one or more of which are concerned ith the etiology of pellegra. To the heat-stable substance he gave ne name P-P factor (pellagra-preventive). This substance now ap- ears to be nicotinic acid. In England the factor became known as itamin B_2, the antineuritic factor being called B_1. In America it as the custom to speak of the antipellagra factor as vitamin G, with ccasional employment of the term P-P factor. The situation was, owever, much more complicated than workers of that time realized.

Kuhn and coworkers ('33), while attempting to concentrate vita- in B_2, isolated from whey an orange-brown dye, now known as boflavin. This substance was announced as representing the most tive preparation of B_2 yet obtained, and probably identical with . It has been shown that this flavin, which in solution gives a strong eenish fluorescence, is an essential nutrient. It appears to have been variably a constituent of extracts containing the B-complex of tamins. It is now known, however, that this dye is not the anti- llagra vitamin and does not prevent the dermatosis produced by rtain faulty diets in rats or chicks, which were formerly believed to the analogues of pellagra in man. It was, therefore, misnamed tamin B_2 or G.

Rat and Chick "Pellagra."—Several dietary regimens were known hich induced in rats a dermatosis that was believed to be "rat llagra." It remained for Birch, György and Harris ('35) to show at the inclusion of maize in these diets prevented or cured the con- ion. Human pellagra has been associated for many years with cessive maize eating, so it is evident that this cereal cannot have a eventive action in human pellagra. Molasses, which is known to deficient in the antipellagra substance, is rich in the factor which res the so-called rat pellagra. The disorders in man and the rat are,

therefore, distinct entities. Birch and coworkers designate th
stance as B_6, a lack of which causes the characteristic dermat
the rat.

Ringrose, Norris and Heuser ('31) first observed a pellag
syndrome in chicks. The preventive factor, they reported, is d
from the antipellagra factor. Lepkovsky and Jukes ('35) foun
the dermatosis of dietary origin in chicks is due to deficiency
entirely different factor (filtrate factor). These authors ('36)
however, that deficiency of riboflavin causes in rats a derm
which is distinctly different from that described by Birch an
workers as due to lack of B_6.

Dam and Schønheyder ('34) and Almquist and Stokstad ('35
scribed a deficiency syndrome in chicks which is characterize
subcutaneous, intramuscular and abdominal hemorrhage an
anemia, due to lack of the fat-soluble factor hitherto unknown.
designate this factor vitamin K. Administration of ascorbic acid
not relieve the condition; hence the hemorrhage is not due
scorbutic state.

There is also a nutritional deficiency disease of chicks describe
Pappenheimer and Goettsch ('34) as nutritional encephaloma
caused by lack of a fat-soluble factor which is present in various
and oils. They have made a 20-fold concentration of the cur
substance by dissolving a fraction of soy bean oil in 95 per
alcohol.

These recent studies on the nutrition of the chick reveal that t
are hitherto unsuspected differences in the nutritive requirement
the mammal and the chick. This is further illustrated by the fact
certain forms of vitamin D, which are highly potent in the pre
tion of rickets in the rat, are of little value for the chick.

Vitamin E.—A striking result of nutrition researches was the d
onstration by Evans and Bishop ('22) that laboratory animals (
mice) could be successfully reared on certain simplified diets of
teins, carbohydrates, fats, mineral salts, and crude vitamins prep
tions, and though apparently healthy adulthood was reached, soon
later such animals exhibited lack of reproductive ability. Their ex
ments showed that very small additions of certain kinds of fo
especially wheat germ or wheat germ oil, would prevent the los
reproductive power. After carefully excluding all known nutrient
responsible for the disability, they announced the existence of a
5th member of the vitamin class which they designated vitami
The early phases of the reproductive process in the female do
require vitamin E. When a curative dose of vitamin E is adm
tered as late as the 5th day after insemination, a few hours be
implantation of the blastodermic vesicles, the normal course of p

nancy is assured. In the absence of this vitamin, the fetus dies after a few days and is resorbed. Neither the ovaries nor ova are affected in this deficiency.

In the male, deficiency of vitamin E causes sterility due to degenerative changes in the germinal epithelium, which resembles those seen in the male deprived of vitamin A. The latter, however, is reparable but the former is irreparable. Not only vitamin E but each of the other well-known vitamins, exerts its own special influence upon reproduction.

Indispensable Unsaturated Fatty Acids.—Although it was learned early in the era of modern nutrition that the body can readily synthesize fat from certain nonlipid substances it was only within the present decade that the indispensability of certain types of unsaturated fatty acids became fully demonstrated. As shown by Burr and coworkers (Burr and Burr, '30) and by Evans and associates (Turpeinen, '38) rats cannot be reared in good health unless the diet contains linoleic acid, linolenic acid or arachidonic acid. These compounds appear to be mutually replaceable. That is, it is only necessary that the diet contain one or more of them. The significance of indispensable fatty acids is obscure as yet.

The Inorganic Elements in Nutrition.—That the body requires calcium and phosporus for the construction of bones was never questioned by physiologists. The appetite for sodium chloride and the richness of body fluids in this compound, the abundance of potassium in all the tissues, the occurrence of iron as a characteristic element in hemoglobin and the presence of sulfur as a constituent of proteins, constituted sufficient evidence that these elements were all essential to life. Magnesium, although relatively abundant in the tissues, played, if any, an unknown role in physiological processes until the present decade when its indispensability was clearly demonstrated by McCollum and Orent ('31). They prepared a diet which was exceedingly low in magnesium. When young rats are restricted to this food they pass successfully through stages of vasodilatation, hyperirritability, trophic disturbances, and fatal tonic-clonic convulsions. The content of magnesium in the blood falls to a low level. This deficiency has been observed in farm animals in several instances.

Iodine was first used in the treatment of goiter by Coindet, who painted goiters with tincture of iodine in 1820. Boussingault, in 1825, wrote "Until now iodine is the only specific known for goiter." In 1895 Baumann (1895) discovered that the thyroid gland contains iodine in amounts easily distinguishing it from other organs. Marine and Williams ('08) showed that deficiency of iodine results in characteristic histological changes in the thyroid gland. In 1916, Kendall ('19) isolated the iodine-containing hormone, thyroxin, from thyroid

glands. In 1917, Marine and Kimball ('17) demonstrated on schoo children the prophylactic value of iodine administered as sodium iodid in Ohio, where simple goiter is endemic. The prevention of goite in man and in farm animals by the provision of iodine is now full established.

Copper had been known as a constituent of the blood of snail since the study of hemocyanin by Harless (1847), but it remained for Hart and his coworkers ('25) to demonstrate that in experimenta anemia produced in rats by restricting them to a diet of milk, the administration of pure iron is not effective in causing restoration o the hemoglobin content of the blood. A small addition of copper with iron resulted in prompt alleviation of the anemia. Copper is, therefore, an indispensable element in the diet.

The nutritional role of manganese has been demonstrated by means of diets very low in their manganese content. Studies with rats and mice have shown that a deficiency of this element in females, results in deficiency of lactation and loss of maternal solicitude for the young, and in male rats it induces complete degeneration of the germinal epithelium (Orent and McCollum '31). It produces congenital debility in second generation young (Daniels and Everson, '35). A definite relationship exists between manganese deficiency and perosis ("slipped tendon") in chicks (Wilgus and associates, '37).

The significance of zinc in nutrition has not yet been definitely established. Certain observations (Hubbell and Mendel, '27; Todd, Elvehjem and Hart, '34) suggest that zinc in the diet of animals stimulates growth and plays a role in the development of a normal coat of hair. Of interest are the findings that insulin, even in purest crystalline form contains zinc (Scott, '34). The indispensability of cobalt in the nutrition of sheep and cattle has been demonstrated (Marston, '35).

Present Concept of Nutrition.—In this brief review of some outstanding discoveries in the field of nutrition we have indicated that in recent decades the principal emphasis has been on the discovery of indispensable nutrients, or the primary components of an adequate diet. We now regard an adequate diet as composed qualitatively of many chemically discrete components provided in such states of combination that they are utilized efficiently. Moreover, the adequate diet must contain these essentials in approximately the proportions required by the body in order to promote optimum efficiency and the prolongation of physiological well-being. Also, the adequate diet must contain a minimum of injurious factors, e.g., selenium and fluorine. Since the necessary experimental procedures are well-defined it appears probable that the next one or two decades will witness an essentially complete solution of the present major nutritional problem.

This is the discovery of all the indispensable nutrients for man and some of the domestic animals. But the field of modern nutrition covers more than qualitative aspects. In addition it is concerned with the determination of the requirements for all nutrients and factors which affect their utilization. Moreover, it involves the study of interconversion and intermediary metabolism of foodstuffs, the chemical reactions concerned with the exchange of energy, and the economical production of nutrients in available form for man and animals. The present state of the newer knowledge of nutrition requires that this book shall be mainly devoted to discussions of specific nutrients as concerns their chemical nature, physiological roles, requirements in the maintenance of health, and the effects produced by their deficiencies. It is this concept of the present problem of nutrition that will determine the scope of the subject matter in the chapters to follow.

REFERENCES

Magendie (1816). Ann. de chim. et de phys., *3*, 66.

Pereira (1843). "A Treatise on Food and Diet." J. & H. G. Langley, New York.

Voit (1881) "Über die Ernährung des Menschen in verschiedenen Klimaten." M. Rieger, München.

Lunin (1881). Ztschr. f. physiol. Chem., *5*, 31.

Pekelharing (1905). Nederlandsch. Tijdschr. N. Geneesk., *2*, 3.

Hopkins (1906). Analyst, *31*, 385.

Takaki (1887). Lancet, *ii*, 86.

Eijkman (1897). Virchow's Archiv. f. Path. u. Anat., *149*, 187.

Grijns (1901). Genesk. Tijdschr. N. Ind., *41*, 3.

Medical Research Committee (1919). "Report on the Present State of Knowledge Concerning Accessory Food Factors (Vitamines)." Special Report Series, No. 38, London.

Stepp (1909). Biochem. Ztschr., *22*, 452; id. (1911), Ztschr. f. Biol., *57*, 135; ib. (1912), *59*, 366.

Funk (1912-13). J. Physiol., *45*, 489.

McCollum and Davis (1913). J. Biol. Chem., *15*, 167.

Osborne and Mendel (1911). "Feeding Experiments with Isolated Food Substances." Pub. Carnegie Inst. of Washington, No. 156, Parts I and II; id. (1913), J. Biol. Chem., *15*, 311; ib. (1913-14), *16*, 423.

Fischer (1906). "Untersuchungen Über Aminosäuren, Polypeptide und Proteïne." Julius Springer, Berlin.

McCollum and Davis (1915). J. Biol. Chem., *21*, 615.

McCollum and Davis (1915). J. Biol. Chem., *23*, 181.

Biggar (1924). "The Voyages of Jacques Cartier." Publications of the Public Archives of Canada, No. 11, Ottawa.

Lind (1753). "A Treatise on Scurvy." Sands, Murray & Cockrane, London.

Medical Research Council (1932). "Vitamins: A Survey of Present Knowl-

edge." Special Report Series No. 167, His Majesty's Stationery Off London.
Holst and Fröhlich (1907). J. Hyg. (Cambridge), *7*, 634; id. (191 Ztschr. f. Hyg. Infekt. Krankheiten, *72*, 1.
Szent-Györgyi (1928). Biochem. J., *22*, 1387.
Waugh and King (1932). J. Biol. Chem., *97*, 325.
Svirbely and Szent-Györgyi (1932). Nature, *129*, 576.
Tillmans, Hirsch and Vaubel (1933). Ztschr. f. Untersuch. Lebensmi *65*, 145.
Mellanby (1918). J. Physiol., *52*, xi; id. (1919), Lancet, *i*, 407; ib. (192 *i*, 856.
McCollum, Simmonds, Parsons, Shipley and Park (1921). J. Biol. Chem *45*, 333.
Sherman and Pappenheimer (1921). Proc. Soc. Exp. Biol. & Med., *18*, 19
McCollum, Simmonds, Becker and Shipley (1922). J. Biol. Chem., 5 293.
Huldschinsky (1919). Deutsche. med. Wchnschr., *45*, 712.
Schmorl (1909). Ergebn. d. inn. Med. u. Kinderh., *4*, 403.
McCollum, Simmonds, Shipley and Park (1922). J. Biol. Chem., *51*, 4
Hess and Weinstock (1924). J. Biol. Chem., *62*, 301; id., Am. J. Dis Child., *27*, 1.
Steenbock and Black (1924). J. Biol. Chem., *61*, 405; ib. (1925), *64*, 263
Hess and Windaus (1926-27). Proc. Soc. Exp. Biol. & Med., *24*, 171.
Rosenheim and Webster (1926). Biochem. J., *20*, 537; ib. (1927), *21*, 111
Smith and Hendrick (1926). U. S. Pub. Health Reports, *41*, 201.
Goldberger and Wheeler (1927). U. S. Pub. Health Reports, *42*, 1299 2383; ib. (1929), *44*, 2769.
Kuhn, György and Wagner-Jauregg (1933). Ber. deutsch. Chem Gesellsch., *66*, 317, 576, 1034.
Birch, György and Harris (1935). Biochem. J., *29*, 2830.
Ringrose, Norris and Heuser (1931). Poultry Sci., *10*, 166.
Lepkovsky and Jukes (1936). J. Nutr., *12*, 515.
Dam and Schønheyder (1934). Biochem. J., *28*, 1355.
Almquist and Stokstad (1935). Nature, *136*, 31; id., J. Biol. Chem. *111*, 105; id. (1936). J. Nutr., *12*, 329.
Pappenheimer and Goettsch (1934). J. Exper. Med., *59*, 35; id. (1936) Am. J. Physiol., *115*, 610.
Evans and Bishop (1922). J. Metabolic Research, *1*, 319, 335.
Burr and Burr (1930). J. Biol. Chem., *86*, 587.
Turpeinen (1938). J. Nutr., *15*, 351.
McCollum and Orent (1931). J. Biol. Chem., *92*, xxx.
Baumann and Roos (1895). Ztschr. f. physiol. Chem., *21*, 319.
Marine and Williams (1908). Arch. Internal Med., *1*, 349.
Kendall (1919). J. Biol. Chem., *39*, 125.
Marine and Kimball (1917). J. Lab. & Clin. Med., *3*, 41.
Harless (1847). Muller's Archiv., p. 148.
Hart, Steenbock, Waddell and Elvehjem (1925). J. Biol. Chem., *65*, 6
Orent and McCollum (1931). J. Biol. Chem., *92*, 651.

Daniels and Everson (1935). J. Nutr., *9*, 191.
Wilgus, Norris and Heuser (1937). J. Nutr., *14*, 155.
Hubbell and Mendel (1927). J. Biol. Chem., *75*, 567.
Todd, Elvehjem and Hart (1934). Am. J. Physiol., *107*, 146.
Scott (1934). Biochem. J., *28*, 1592.
Marston (1935). J. Council Sci. Ind. Research, Australia, *8*, 111.

CHAPTER

III

Carbohydrates in Nutrition

THE OUTSTANDING importance of sugar metabolism as a source of energy in the animal body, and great commercial importance of the fermentation industries, make the chemical processes brought about in biological transformation of the sugars one of the attractive fields for investigation by biochemists. This interest is heightened because of the frequency of perversion of sugar metabolism, as for example, diabetes in man. The result is an enormous output of papers dealing with a great variety of aspects of carbohydrate metabolism. No adequate discussion of this general subject is within the province of this book, but a selection of certain of the more interesting newly-discovered facts has been included because they are so fundamental to an understanding of physiological well-being as it is influenced by carbohydrate utilization.

Rate of Carbohydrates Absorption.—Cori and Cori ('28) state that different sugars are not absorbed at the same rate. Thus if the rate for glucose is taken as 100, the rate for galactose, fructose, mannose, xylose, and arabinose are, in the order given: 110, 43, 19, 15 and 9. All of these sugars, when introduced into the peritoneal cavity, are absorbed at the same rate. This shows that the intestine exerts a highly selective action on carbohydrates and that this action is not shared by another membrane, i. e., the one lining the peritoneum. When two sugars are in solution together in the intestine and are absorbed simultaneously, the rate for each is reduced. Thus when glucose and galactose are fed in mixture, the total amount of sugar absorbed is not larger than when each sugar is fed separately. This rule was found by Cori ('26) to hold true not only when two sugars were fed together, but for two amino acids, or one sugar and one amino acid. The rate of absorption of sugar is not increased, he states,

when increased amounts are ingested. Only a limited number of molecules seem to be able to pass through the intestinal mucosa in a given time. This does not hold for alcohol, which is absorbed the more rapidly the higher the concentration in the intestine. It seems probable, however, that the amount of sugar absorbed in a given time would be much greater when a larger volume of a sugar solution of a given concentration is taken, than in the case when a smaller volume is taken, since it would spread over a larger area of mucosa, each area absorbing at the normal rate. This assumption seems necessary since earlier observations demonstrated that different individuals showed decided differences in the concentration of glucose in the blood when 100 gm. of this sugar were taken in solution under comparable conditions. Groen ('37) studying carbohydrate absorption in man, found that each sugar has its individual absorption rate.

Intolerance for Carbohydrate after Fasting.—Goldblatt and Ellis ('32) state that after a period of some 40 hours of complete starvation in human subjects, the administration of a dose of glucose, normally easily tolerated, was followed by high and prolonged elevation of blood sugar curves, glycosuria, and a smaller rise in the R. Q. than that occurring in ordinary post-absorptive conditions. This intolerance, they find, is not due to either ketosis or acidosis. It is in line with the observation of Dann and Chambers ('30, '32) that in dogs, after a period of starvation, insulin does not completely reestablish normal carbohydrate oxidation. Goldblatt and Ellis ('32 l.c.) express the view that in the starving organism there is a temporary dissociation of the two mechanisms initiated by insulin, viz., the formation of peripheral glycogen, and the oxidaton of carbohydrates, the second effect being manifest only when the first has attained adequate proportions. But in view of the specific role of thiamin in carbohydrate metabolism (Chapters XVIII and XIX) it is plausible to suppose that in these experiments the subjects were suffering some degree of thiamin deficiency.

Glucose, Galactose, and Lactose as Glycogen Formers.—Deuel, MacKay, Jewel, Gulick and Grunewald ('33) found that when glucose was administered to female dogs previously fasted for 6 days the accumulation of glycogen was greater 6 hours after ingestion than in animals which retained a corresponding amount of galactose. However, the liver glycogen in animals killed after 12 and 72 hours after sugar feeding was higher in dogs which had taken galactose than in those which had taken glucose. After an interval of 6 and 12 hours from feeding, the muscle glycogen was higher in the glucose-fed dogs. In a long series of tests with rats, it was found that when the animals had been fed diets high in galactose, lactose, or galactose-glucose for 7 to 12 days, the liver and muscle glycogen were almost invariably

higher after periods of fasting up to 72 hours than they wer animals were fed a similar diet containing glucose only as th hydrate. They suggest that the glycogen formed from gala retained longer than that formed from glucose, although in ments on dogs it appears that glycogen is formed faster from These results afford an explanation for the superior ketolyti which the authors had previously reported for galactose in subjects as well as in fasting rats in which a mild ketosis is art induced by the oral or subcutaneous administration of diaceti Feeding rats a high-galactose diet over a period of 3 months increase the well-known low tolerance for galactose above t rats kept for the same period on a glucose-containing diet.

Bell ('35, '36) applied the method of "end-group assay" d by Haworth and Percival ('32) to the glycogen from livers of r which had been fed galactose. The "galactose glycogen" mo consists of a chain of 18 glucose units whereas the glycogen fron mally-fed rabbits which is derived from glucose consists of 12 g units. Young ('36), using the methods of Bell and Young ('34 , that the liver and muscle glycogen were identical. The specific tory power of glycogen from the two sources was likewise tially identical. The manner of feeding the rabbits from whic glycogen was obtained was not stated, but it would appear tha glycogen from both of these sources was derived from glucose.

Deuel, MacKay and Gulick ('32) studied the quantitative tionships in glycogen storage in rats' livers and muscles upon ing glucose, galactose, and lactose respectively, following fasting riods of 24, 33, 48, and 54 hours. The glycogen content of both ti of galactose-fed rats far exceeded those of glucose-fed animals a end of these fasts. Rats fed galactose after previous fasting periods higher values for liver glycogen if they had been previously galactose than if they had been previously fed glucose. Lactose ing gave intermediate values as compared with glucose and galac It appears, therefore, that notwithstanding the low tolerance of body for galactose, and the tendency for this sugar to pass thr the kidneys, it is a good glycogen former. The glycogen formed it persists much more during fasting than is the case when gluco fed.

Dann and Chambers ('30 l.c.) showed that a complete, th temporary, loss of the ability to burn glucose occurs in dogs after a of three weeks or more, with frequent exercise on the tread During the first four hours after 50 gm. of glucose were inge the blood sugar rose to a high level and a part of the sugar excreted in the urine. However, at least 30 gm. could not be counted for either by excretion or oxidation. In a later study ('32

they determined the glycogen, glucose, and lactic acid in portions of liver and muscles of dogs subjected to fast and then given glucose. They found that the power to form glycogen was not seriously impaired after a fast sufficiently long to suppress almost all sugar oxidation. Absorption of 25 gm. of glucose from the alimentary tract was practically complete within 4 hours in these animals. MacKay and Bergmann ('33) conclude from similar studies on the rat that the variations in blood glucose tolerance curves caused by feeding glucose after 24-48 hours' fasting are due, in large part, if not entirely, to changes in the rate of glucose uptake by tissues other than the liver. These changes, they believe, are probably produced by impairment of their capacity to oxidize glucose. Here, also, the possibility should be considered that the animals were suffering some degree of thiamin deficiency.

Murlin and coworkers ('36) found that in human subjects, the ketosis produced by taking high fat diets was greatly reduced but never quite extinguished within 3 to 4 hours by feeding sugars. Sucrose is somewhat more effective in doses up to 60 gm.; glucose is more effective in doses of 100 gm. Fructose stands between the other two. Although the quantity of carbohydrate oxidized by these subjects was more than sufficient, judging by the Shaffer theory, which maintains that the combustion of one molecule of glucose will cause the combustion of two molecules of aceto-acetic acid, the ketosis was not completely controlled. In only one of nine subjects was the change in ketogenesis in exact agreement with the increased combustion of carbohydrate. Glycogen formation seems to be as important as combustion in the antiketogenic action of sugar. These investigators suggest that the presence of a certain minimal "normal" content of glycogen in the liver is a condition precedent to the normal oxidation of fatty acids. There is somewhat more evidence, however, that this normal content of glycogen prevents the formation of ketone substances in the liver.

Deuel, Gulick and Butts ('32) insist that galactose is markedly superior to glucose in preventing the formation of ketone acids, which result from fatty acid oxidation when oxidation of sugar is impaired. This is surprising in view of the well-known fact that galactose is more difficult to metabolize than glucose, sucrose, or fructose, and its ingestion is attended by greater tendency to hyperglycemia and glycuresis than is the case with the other sugars. Goldblatt ('25), working with human subjects subjected to a 36-hour fast of both food and water, concluded that glucose, fructose, sucrose, and maltose were ketolytic, but that galactose, mannose, lactose, and glycerol exerted no such effects. Deuel and coworkers question whether his tests for acetone bodies, which were qualitative rather than quantitative, af-

forded sufficient data; they also question whether his method of paring his subjects for study gave a sufficiently uniform level of ket from which to start his experiments.

The Conversion of Carbohydrate into Fat.—Wesson ('27) Wesson and Murrell ('33, '34) have reported the results of stu which appear to show that in the absence of an unidentified diet factor there is, in rats, an abnormally rapid conversion of carbohydr into fat. They restricted rats for 2 or 3 weeks to a diet deficient fats and very rich in carbohydrate. In some experiments the anim were restricted to dextrin, calcium carbonate, and water. These r were then given a definite amount of dextrin and their respirato quotients measured. When carbohydrate alone is being metaboliz the R. Q., $\frac{\text{vol. of } CO_2}{\text{vol. of } O_2}$, is I. It is well known that when carbohydra is being converted into fat an oxygen-rich substance is being co verted into an oxygen-poor one, and the R. Q. is greater than 1. The found respiratory quotients in their rats which had been long r stricted to a fat-free regimen to be from 1.5 to as high as 2, whic indicated that they were converting carbohydrate into fat at an ab normally high rate. The administration of small daily amounts (6.6 mg.) of either the liquid or solid fraction of lard prevented th metabolic abnormality although the stunting and emaciation were o about the same degree as in the control animals on the fat-free regimen The liquid fraction was more than ten times as active as the solic fraction. This abnormal fat formation is not brought about by reason of a need for fat for catabolism but because of deficiency of some metabolic factor which is supplied by certain fats. Ethyl stearate wa inactive. They were unable to secure the effects mentioned with an known vitamin or any of the essential amino acids. Lineoleic acid wa not effective even when fed in large amounts and over long periods The fatty acid fraction obtained from saponified lard was active, hence the hypothetical substance, in the absence of which abnormal fa formation takes place, is stable to saponifying agents.

Wesson and Murrell ('33 l.c., '34 l.c.) have extended their studie to include restriction of rats to (1) sucrose, (2) maltose, (3) lactose (4) d-glucose, (5) d-fructose, (6) d-galactose, (7) d-mannose, (8) l-arabinose, (9) d-xylose. The sugars containing d-glucose (1-4) and d-xylose (9) gave R. Q. curves appreciably higher in the abnorma rats than in the normal ones. The remaining carbohydrates (5-8) gave values practically the same for both abnormal and normal rats.

Pentose Metabolism.—It has long been known that the tolerance of humans and of mammals, generally, for the pentose sugars is very low. Since the pentosans, which are very abundant in many vegetable foods, are indigestible in the alimentary tracts of the Omnivora and

Carnivora, and since pentose sugars occur in natural foods in but small amounts, these carbohydrates have been of little importance in the nutrition of man. Miller and Lewis ('32) call attention to the fact that in 1930 the U. S. Bureau of Standards announced a simple method for the preparation of xylose from cottonseed hull bran, which is cheap and abundant. Xylose is now obtainable in large amounts and at a price comparable with that of sucrose. The commercial possibilities of exploiting xylose did not escape the notice of the unscrupulous, who have brought it to the attention of the public as a "slenderizing sugar from cottonseed," and as a "non-fattening sugar." Miller and Lewis, as a result of their new studies on the nutritive value of pentose sugars, conclude that although xylose is absorbed fairly readily from the digestive tract, no evidence could be secured that it is at all utilized in the nutrition of the rat. Rhamnose, a-methyl pentose, is absorbed more rapidly than is d-xylose, but it is not utilized by the body.

Role of the Hypophysis.—The hypophysis has been shown to exert a regulatory action on the blood sugar and on glycogen formation. Thus, Russell ('36) found that hypophysectomized rats, when well-fed show normal levels for blood sugar, and liver and muscle glycogen. When fasted, these levels fall extremely rapidly as compared with normal animals. The figures in the following table represent the decrease in carbohydrate content as per cent of the average levels found in well-fed animals.

DECREASE IN CARBOHYDRATE LEVELS DURING FASTING OF NORMAL (N) AND HYPOPHYSECTOMIZED (H) RATS

	8-HOUR FAST		18-HOUR FAST	
	N	H	N	H
Liver glycogen	27	95	96	99
Muscle glycogen	8	24	6	41
Blood glycogen	20	49	32	54

Rats used as controls, which were subjected to operation in which one-third or more of the anterior lobe of the hypophysis remained, including several with various degree of brain injury, did not differ from the normal and were not included in the above table. In operated rats, after fasting, the feeding of starch causes a restoration of muscle glycogen and blood glucose to normal, but liver glycogen values increase much more slowly than in normal animals. The rates of digestion and absorption of ingested carbohydrate were shown to

be about 30 per cent below normal in the operated rats. Fisher Pencharz ('36) found that a larger proportion of absorbed carb drate is oxidized by hypophysectomized than by normal rats follow glucose feeding. This may account for the decreased amounts st as glycogen in the liver.

Russell and Bennett ('36) found that intraperitoneal injection hypophyseal extracts were very effective in preventing the rapid ing off in the glycogen stores of well-fed rats during early fasti They tested an extract containing all the known hormones of hypophysis, two extracts that were rich in the growth hormone a one which had a high content of the adrenotrophic hormone and c taining a fairly large amount of growth hormone. All were effecti They used preparations from which the gonadotropic and mamm tropic hormones had been removed and found these still effective protecting the glycogen stores. One active preparation appeared contain no thyrotropic hormone. It appears that these hormones m be ruled out in connection with the effect of the hypophysis glycogen stores. Working with adrenalectomized rats these autho seem to have excluded the possibility that the hypophyseal extrac exert their influence through the adrenal cortex. Boiled preparatio were without effect. Russell and Bennett state that muscle glycoge is the store principally affected. It has been shown by Fisher, Russe and Cori ('36) that rats in the hypophysectomized state, which wer losing muscle glycogen had correspondingly higher respiratory quo tients than normal animals. Treatment with the same type of extract as were used by Russell and Bennett, which diminished the loss o muscle glycogen, restored the respiratory quotients to the levels ob served in normal rats. The hypophysectomized rats excreted the sam amount of nitrogen during the experimental period as the norma animals. It is concluded that hypophysectomized rats suffer a defec in the mechanism by which normally carbohydrate oxidation is de pressed, and carbohydrate levels are thereby maintained during fasting and that anterior lobe extracts are able to restore this function, ap parently by exerting a depressing effect on carbohydrate oxidation

Role of the Adrenal Cortex.—The adrenal cortex is also inti mately associated with the regulation of the storage of glycogen i the tissues. But whereas the hypophysis is shown to exercise a regu latory function on the rate of oxidation of carbohydrate, the effec of the adrenal cortex seems to be largely concerned with synthesi of glycogen, a function which is lost in the adrenalectomized animal Evans ('36) showed that fasting adrenalectomized rats failed to re spond to lowered atmospheric pressure with increased deposition o glycogen in the tissues as is the case in normal animals. This ha been traced in the adrenalectomized rat to failure to metabolize addi

tional protein under reduced atmospheric pressure. The normal rat, when placed under one-half an atmospheric pressure, excretes an excessive amount of nitrogen which is derived from tissue destruction. The adrenalectomized rat does not do so. On the average, about 58 per cent of the carbon of protein can be converted into glucose and is so converted when there is a carbohydrate deficit. In phlorhizinized animals whose renal epithelium is altered so as to reduce the threshold of sugar elimination to zero so that sugar leaks away rapidly, the glucose and nitrogen excretion are profoundly reduced. This situation does not arise when the medulla of the adrenal alone is extirpated, hence the effect is referred to the cortex. Evans was unable to restore normal protein-metabolizing power to adrenalectomized rats by administration of extracts of adrenal cortex.

Buell, Anderson and Strauss ('36) have observed that in adrenalectomized rats liver glycogen formation from d-lactic acid is greatly reduced. Administration of the active principle of the adrenal cortex in the form of the charcoal adsorbate of Grollman, Firor and Grollman restored the power of forming glycogen from this acid. Butts et al. ('35-36) demonstrated that normal animals can readily form glycogen from d-alanine, and Samuels, Butts, Schott and Ball ('36-37) have shown that this amino acid is not well utilized for glycogen formation in adrenalectomized rats. It seems not to have been determined whether this failure is due to inability to convert alanine to sugar, but this is to be inferred since Samuels and coworkers found low blood sugar values. Butts has demonstrated that d-alanine, but not the l-isomer, is an excellent glycogen-forming substance. Barnes and Regen ('34) have found that dogs made hypersensitive to insulin by denervation of the adrenals or by hypophysectomy can be protected against two to four times the convulsion-producing dose of insulin by a simultaneous injection of epinephrine at a constant rate.

Sex Variation in Carbohydrate Metabolism.—Deuel and Gulick ('32) and Deuel and coworkers ('33) have called attention to the fact that normal women excrete much larger quantities of acetone bodies during fasting than do normal men, and they have also shown that similar discrepancies occur between male and female guinea pigs and rats during inanition when these animals are fed sodium acetoacetate. The characteristic behavior in this respect suggests that there must be a fundamental difference in the carbohydrate metabolism of the sexes. Greisheimer ('31) first pointed out that when fed various diets male rats had higher liver glycogen and lower liver fat content than did female animals on similar diets. Others have provided analytical data which confirm this fact. Stohr ('32) confirmed these findings with young, but not with old rats of unstated ages. Deuel and coworkers ('32, l.c.; '33, l.c.) have found that the liver glycogen

was significantly higher in male than in female rats 24, 48, and hours after feeding glucose. The liver fat was higher in female th in male rats during the entire fasting period. When rats were a high carbohydrate diet and were killed without fasting there v no difference in the glycogen content of the livers of the sexes. T liver glycogen of female guinea pigs was higher after 48 hours' f ing than in males, but the liver fat content (lipid) in males a females corresponded with the relations found in rats. Deuel poi out that the experiments on guinea pigs were made on animals undetermined ages. The amount of diacetic acid which is oxidized fasting male rats is considerably higher than that utilized by fema rats when it is administered orally.

Gulick, Samuels and Deuel ('34) found double the amount glycogen in the livers of ovariectomized rats as compared with norm females 48 hours after feeding comparable amounts of glucose base on estimated surface area. When theelin was administered to ovarie tomized rats, lower values were obtained from them than from uni jected controls. They do not, however, believe that the sex differenc in carbohydrate metabolism is solely dependent on the production o theelin.

Recent observations, therefore, demonstrate clearly that not onl the pancreas, through its production of insulin, influences carboh drate utilization, but that this is influenced by the hypophysis, adrenals and sex glands as well. The influence of the thyroid gland on meta bolic rate is, of course, well known.

For years it has been a matter of controversy whether the vagu nerve controls the activity of the pancreas in the secretion of insulin or whether the response of that organ is regulated by the concen tration of sugar in the blood. The discussion still continues, but i now seems to be fully established that the blood sugar level is th normal regulating mechanism. Gayet and Guillaumie ('27, '33) trans planted the pancreas of dogs to the neck by means of vascular anasto moses. Stimulation of the vagus nerve caused copious external pan creatic secretion, but there was no lowering or other change in th blood sugar. Injection of hyperglycemic blood into the artery of th transplanted pancreas caused a precipitous drop in the blood sugar Kosaka ('33) showed by infusing a glucose solution into the pan creatoduodenal artery, the femoral artery, and the portal vein, tha only in the first case was there a pronounced drop in the blood sugar This appears to prove that the pancreas responds to an increase blood sugar concentration in its arterial blood with increased liberatio of insulin.

The Nutritive Value of Carbohydrates.—Following the inges tion of starch there is almost as rapid a rise in blood sugar as whe

glucose is taken, hence starch must be hydrolyzed to glucose very rapidly. Quite characteristically, however, there is no rise in the blood sugar content after taking even considerable amounts of milk sugar or lactose. The questions arising from these observations are: Is failure of lactose to elevate the blood sugar due to delayed absorption dependent upon slow hydrolysis, or to the rapid passage through the alimentary tract with consequent slow absorption? The fact is well established that a considerable amount of lactose may reach the colon, and because of its presence there lead to the establishment of *L. acidophilus* as the prevailing flora, and consequent depression of proteolytic types of bacteria. Lusk ('15) showed that lactose in the dog failed to produce the specific dynamic effect shown by glucose or sucrose and failed to materially increase the respiratory quotient. It has long been known that lactose injected intravenously or subcutaneously is excreted in the urine unchanged.

Deuel and Chambers ('25) found that in the phlorhizinized dog, after feeding lactose, about 50 per cent appeared in the urine as glucose. The rat appears to be more capable of utilizing lactose than is the dog. Greisheimer and Johnson ('30) demonstrated that glycogen is formed from lactose in the rat, but after 16 to 18 days on diets deriving 87.5 per cent of their caloric value from sugar, the liver glycogen amounted to 62 per cent of that found in animals fed glucose or sucrose. H. S. Mitchell ('27) found that growth on diets containing 30 per cent lactose was only about 50 per cent as great as that of rats fed other carbohydrates in comparable diets. She found that the amount of carbohydrate lost in the feces of these rats was only 3.12 per cent greater in the lactose animals than in those fed sucrose. Both the average food intake and voluntary activity decreased in the lactose period as compared with the glucose period, hence the decreased rate of growth was not due to expenditure of energy in work. The possibility that increased hydration of the tissues entered into the differences between lactose- and glucose-fed rats was ruled out by experiment. Koehler and Allen ('34) conclude from their experiments that of ingested lactose, approximately 40 to 50 per cent may be lost to the rat as far as weight or energy relationships are concerned. A part of the ingested lactose is converted through fermentation by *L. acidophilus* organisms in the gastrointestinal tract into lactic acid, but this when absorbed behaves like carbohydrate in metabolism. Subsequently it will be pointed out that the increased acidity in the intestine caused by the ingestion of lactose favors the absorption of calcium. In chick feeding it is generally believed that lactose in the digestive tract is effective, presumably because of increasing the acidity, in holding in check the development of coccidiosis.

H. S. Mitchell's ('27, l.c.) results on growth of feeding lactose

have not been confirmed by others. Thus Whittier, Cary and Ellis ('34) compared lactose fed to young rats at the level of 45 per cent of the diet, with sucrose and dextrin fed in similar amounts. They observed that during the early period rats given lactose increased in weight much faster than did those receiving sucrose. After reaching adult age the sucrose-fed rats became heavier than the lactose-fed animals. The former consumed more food than did the latter. The differences in weight of the adults was accounted for on the basis of the greater amount of fat in the bodies of the sucrose-fed rats. These results were confirmed with swine which accumulated fat faster when fed sucrose than when fed lactose. The lactose-fed rats lived longer than did those on the sucrose diet. Feeding excessive quantities of lactose to rats caused diarrhea for a short time; this retarded growth, but this disturbance was soon overcome. They conclude that the characteristic results obtained in their experiments were not the result of stimulation of acidophilic organisms in the lower intestine since dextrin accomplishes this result; but it does not have the same influence on growth as does lactose. These results are in harmony with the view expressed by Jarvis ('30) that "age-weight for age-weight, the lactose-fed infant possesses more living tissue than does the infant fed on vegetable sugar."

Feyder ('35) studied the nutritive value of dietary sucrose and glucose. Under the conditions of his experiment the sucrose diet resulted in more rapid gains in weight than the glucose diet due to a more rapid deposition of fat and also a markedly greater deposition of protein. The glycogen formation was practically the same in the two sets of animals.

H. H. Mitchell ('37) compared the value of glucose and sucrose, glucose and fructose, and glucose and lactose in the diet. He found that in case of sucrose, fructose, and lactose intake, the digestibility of the organic nutrients of the diets became impaired, being greater on the lactose, intermediate on the fructose, and least on the sucrose-containing diets. Growth was affected depending on the degree of impairment. The metabolic utilization of calcium was increased by sucrose, fructose, and lactose. Furthermore, lactose definitely furthered the utilization of phosphorus.

The Effects of Lactose in the Diet.—Dragstedt and Peacock ('23) were the first to call attention to the fact that the inclusion in the diet of 50 to 100 gm. of lactose daily would prevent the onset of tetany in thyroparathyroidectomized dogs. Feeding lactose has been shown to modify the intestinal flora toward the aciduric rather than the proteolytic type. Dextrin has been reported to possess this property in some degree whereas other carbohydrates seem to be without influence on the intestinal flora. Hudson and Parr ('24) found that

lactose, in contrast with other carbohydrates, produces an aciduric flora, and consequently an acid reaction in the intestine. The prevention of the onset of tetany in animals deprived of the parathyroid glands which exert a regulatory influence as a result of feeding lactose is ascribed to the increased facility of the intestinal mucosa owing to the acid reaction in the intestine in absorbing calcium, since, as is well known, tetany can be alleviated by the administration of soluble calcium salts.

Kline and his coworkers ('32) observed that 40 per cent of lactose included in the ration of chicks fed a rickets-producing diet had a favorable effect upon calcium absorption and skeleton building. There was maintained throughout the entire length of the intestinal tract an acid concentration greater than was produced by the basal ration containing other carbohydrate. When but 20 per cent of lactose was fed, calcification was improved to a lesser extent than with 40 per cent, and with 2 to 10 per cent the effect was slight. Feeding 40 per cent of maltose, or 5 per cent of citric acid, produced no effect on the intestinal reaction or calcium absorption. Inouye ('24) states that feeding galactose exerts an influence similar to lactose feeding on the prevention of the onset of tetany in dogs.

Barenberg and Abramson ('30) studied the effect of including large amounts of milk sugar in the diet of infants. The ingestion of 12 to 15 per cent of the diet in the form of milk sugar did not cause loose stools. Infants fed 15 to 17 gm. of lactose per kilogram of weight, in health or disease, did not excrete sugar in either urine or feces. Few acidophilic organisms were found in the stools. The infants receiving large amounts of milk sugar responded with a better rate of growth than did the control group. They conclude that milk sugar is a safe carbohydrate for the modification of cow's milk for infant feeding, and that it does not have a laxative effect in infants when used even in high concentrations.

Gerstley ('30) has reviewed the history of lactose in infant feeding and notes that every text-book on infant feeding reflects a fear by pediatricians that lactose as a sugar for modifying cow's milk for infants is attended with danger. He attributes this fear to clinical experience plus uncritical acceptance of the teachings of Escherich (1886). Following the favorable reports on the use of lactose in infant feeding by Barenberg and Abramson (l.c.), Gerstley employed this sugar in his practice and is convinced that where diarrhea and intestinal fermentation arise, they are due to some other element in cow's milk than lactose. He finds that lactose added to whole boiled cow's milk does not result in diarrhea with increase in the excretion of acids when given to normal infants. The amount of various acids excreted by the intestine does not depend directly upon the amount

of lactose in the diet but on various factors which are probably
cerned in its absorption from the intestine. The relation of la
to protein in the infant's diet is of great importance. In respe
certain chemical relationships in the stool, lactose, he believe
preferable to maltose-dextrin preparation.

Kopeloff and Cohen ('30) express similar views. They point
that the normal nursling thrives on a lactose-rich diet. Lactose i
much a food for beneficial microbes as for man. In the intest
flora of the breast-fed baby *Bacillus bifidus* predominates. When
baby is weaned to cow's milk, *L. acidophilus* becomes the
dominating microbial species. Lactose, as is well known, is an excel
source of energy for this microbe. They emphasize that lactose sti
lates the acidophilus bacilli native to the individual's intestinal t
which is preferable to the ingestion of alien strains. Its slow abs
tion, which enables much of the ingested sugar to reach the co
where it serves as a pabulum for *L. acidophilus,* is a valuable prope
Their experimental observations show an effective transformation
the colon flora to the *L. acidophilus* type in only about 20 per c
of their cases when 100 gm. of lactose were fed daily. The ingesti
of 50 gm. daily appeared to be as effective as the larger intake.

The cause of the appearance of lactose in the urine of pregna
and lactating women has been studied by Watkins ('28). He sta
that during the last stages of pregnancy there is a more or less co
stant excretion of small amounts of lactose, and that this phenomen
seems to be in no way related to the activity of the mammary glan
During the last days before delivery there is a sudden very mark
rise in lactose excretion which reaches its height on the day
delivery. After delivery the lactose excretion immediately drops to
low level for 2 to 5 days. There is then a sudden and often t
mendous excretion of this sugar, and during the first few weeks
lactation, the fluctuations in its excretion are very great. By the e
of the first month after delivery the lactose excretion has assum
a constant and lower level and slowly approaches the normal valu
as lactation progresses. The tolerance of most women for lactose
in the intermenstrual period, the same as that of normal men, t
tolerance dose being about 10 gm. Menstruation causes an increa
in the tolerance of women for lactose so that many women at th
time show no urinary responses to the ingestion of 20 gm. of t
sugar. During pregnancy the tolerance for lactose is increased, reac
ing in some cases three times that of non-pregnant individuals. Duri
lactation the tolerance for this sugar is about the same as in norm
men and in most women in the intermenstrual period.

Galactose in the Diet.—Galactose originates almost entirely in t
lactose of the diet and is, therefore, a more prominent digestive pro

uct in infants than in adults. Galactose is absorbed slightly more rapidly than glucose. It is only a temporary blood component. According to Cori ('25) galactose does not play a significant role as a source of liver glycogen. However, galactose may perform an important function in the processes involved in the early growth of the child. This carbohydrate is a component of the glycolipins which are structural components of nervous tissue. It is believed that the adult is independent of galactose in the diet and can synthesize this sugar at a rate adequate to replace the losses incurred through wear and tear. This may not be true of the newly-born infant. According to Mathews ('25) a rapid production of glycolipins takes place during the first six weeks of postnatal life. He suggests that the large amount of lactose in human milk may be related to these synthetic changes. If cow's milk is used as a basis for the infant's diet, the supply of galactose will be ample. However, in the infant's dietary where modification of cow's milk is used, the presence or absence of galactose may assume great importance.

Cataract in Rats Fed High Lactose and High Galactose Diets.— H. S. Mitchell and Dodge ('35) made the observation that rats quickly develop cataract when fed a diet containing 70 per cent of lactose. Rations containing less lactose caused slower development or less pronounced lens changes. Cataracts never appeared in rats fed starch, maltose, dextrin, or sucrose, respectively, as the sole source of carbohydrate at the 70 per cent level. The developing opacities give the histological picture of human cataract. Blood, urine, and tissue analysis indicated some disturbance in both carbohydrate and mineral metabolism. Galactose was found in the urine. The calcium content of cataractous eyes was double the normal, but the total and diffusible calcium of the blood was not significantly altered. The inclusion in the diet of 35 per cent of galactose likewise induces cataract in rats. This corresponds to the amount of galactose in a diet containing 70 per cent of lactose. It is, therefore, the galactose part of the lactose molecule which causes the disturbance. P. L. Day ('36) has confirmed these observations. Seventeen of 18 rats receiving 60 per cent lactose developed cataract at an average time of 44 days. Three rats receiving the same amount of galactose exhibited cataract on the 11th day. Glucose, sucrose, and starch did not cause cataract. The mean level of blood sugar in rats receiving glucose or starch was 121 mg. per 100 cc. The mean level of 89 determinations of sugar in lactose-fed rats was 160 mg.; the highest value was 234 mg. The mean of 27 determinations upon galactose-fed rats was 372 mg., the highest value being 556 mg. These results are of great interest because of the frequent occurrence of cataract in human subjects with diabetes. As yet it is undetermined whether it is caused by persistent

hyperglycemia, by lipemia, or by some other factor. P. L. Day a
others have described the experimental production of cataract in r
by depriving them of riboflavin. He states that cataract caused
these two procedures is easily distinguished with the ophthalmosco
Since in the vitamin deficiency cataract there is no elevation of blo
sugar, it is still an open question whether hyperglycemia is the cau
tive factor.

H. S. Mitchell ('36) found that rats from different colonies f
the lactose or galactose cataract-producing rations showed variab
susceptibility to cataract. Young rats were more susceptible than old
ones. Yudkin and Arnold ('35) have confirmed these observatio
and pointed out that in young rats the changes take place in th
growing areas of the lens, the nucleus being altered, whereas in old
rats the periphery of the lens is involved. In these experiments the to
erance for galactose was greatly exceeded. There is no evidence tha
the amounts of lactose which are likely to be employed in modific
tion of cow's milk for infant feeding would produce any untowar
effects on health.

H. S. Mitchell, Cook and Merriam ('37) report that the catarac
producing action of lactose or galactose rations is not appreciabl
altered by:—inclusion as supplementary carbohydrate, starch, dextrin
sucrose, or glucose; substituting different proteins such as casein, drie
meat, egg albumen, or lactalbumin; changing the source of fats by
including respectively, Crisco, butter, mutton tallow, or cod liver oil
addition of excess of cholesterol; feeding different amounts of the
salt mixture (0.4 and 10 per cent); including excess of calcium lactat
(4, 6, and 8 per cent); shifting the acid-base balance (4.76% Na
citrate or 3.4% ammonium chloride); the amount of water in
gested; administration of vitamin C; deficiency or excess of B_1 (thia
min) and B_2; ingestion of large doses of dinitrophenol; or injection
of lens antigen. Cataract-producing action was hastened by lowering
the protein intake to 5 per cent and it was slightly delayed by addition
of cystine.

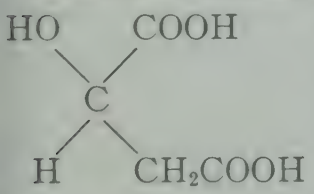
Relation of Carbohydrates to the Phenomenon of Refection.—
Refection refers to a condition in experimental animals under which
they are able to grow and reproduce, even in the second generation
on diets free from the vitamin B complex. The condition was firs
observed by Fridericia ('26) and independently by Roscoe ('27)
Bliss ('35), Guerrant and coworkers ('35), and Guerrant and Brown
('37) have made important contributions to the understanding o
this phenomenon. Bliss finds that refection can be easily produced
by feeding diets containing raw potato starch as the carbohydrate
He carefully excluded the possibility that this starch is contaminated
with the vitamins. Cooked potato starch does not cause refection. In

refection the feces are bulky, white, and gas-occluded. Feeding corn or rice starch did not produce such feces, nor did it promote nutrition such as is caused by refection.

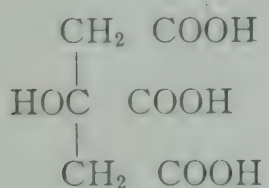
Guerrant and associates ('35 l.c., '37 l.c.) found that dextrinized corn starch exhibited the unusual dietary effect of producing refection. They have shown that the elaboration of the vitamin complex takes place in the cecum of the rat and suggested that this is due to the action of certain microorganisms, and is the result of delayed digestion of the dextrin so that it reaches the cecum in amounts sufficient to afford a medium for the growth of these organisms. They found that it was essential that the rats ingest their feces in order to obtain the full effect of the synthetic products referable to the flora of the digestive tract. Since yeast cells were found in enormous numbers in the ceca of refected rats, it is suggested that the synthesis of the vitamins is the result of their profuse growth in the cecum under conditions which provide a favorable medium for their proliferation.

Kelly and Parsons ('37) found that gelatinization of potato starch at temperatures not significantly destructive of thiamin prevented the occurrence of refection. On uniform intakes of a ration high in potato starch, rats tended to eliminate about three times as much total vitamin B in the feces when the starch was raw as when it was gelatinized. The non-extractable fat of the starch is not a significant factor in the phenomenon of refection.

The Nutritional Significance of Certain Organic Acids.—*Malic acid* occurs widely distributed in apples, pears, peaches, tomatoes, sugar beets, rhubarb, maple sap, alfalfa, plums, quinces, strawberries, citrous fruits, etc. A considerable part of the acidity of many fruits and vegetables is due to this acid. It serves as a source of glycogen, hence it is a source of energy to the body. It is essentially non-toxic and is a wholesome nutrient. It is easily oxidized in the body, combustion being complete to the end-products water and carbon dioxide.



Citric acid is even more widely distributed than malic acid. Indeed, it seems to be ubiquitous, occurring in cereal grains, milk, and other animal products, as well as in a great variety of fruits and vegetables. It is said to be absent in oysters, clams, crabs, shrimps, and scallops, but these may contain traces difficult to estimate. Milk contains 1.0 to 4.0 gm. per liter. The amount daily excreted in the urine of a man amounts to 0.2 to 1.0 gm. It has been



detected in almost every tissue and fluid in the body. It is so metabolized that little extra citric acid is excreted in the urine large amounts of citrous fruit juices are taken. Blatherwick and ('22) found that the citric and other organic acids in 2400 orange juice taken in one day by human subjects was almost pletely destroyed by oxidation. There is some evidence that citri may be converted into glycogen.

Sherman, Mendel and Smith ('36) confirm the findings of O ('31) that the amount of citrate excreted by an individual directly with the pH of the urine regardless of the cause o alterations of this value. The addition of sodium bicarbonate (1 cent of the dry mixture) to the basal citrate-low diet of rats c a 100-fold increase in citrate elimination. They studied citrate e tion in dogs fed high-protein low-carbohydrate, and low-protein carbohydrate diets and found no consistent changes in citrate excr The ingestion of the basal, citrate-low diet generally produce increase in the rate of citrate excretion, and in some but not all a slight rise in the blood citrate concentration in dogs. In dog administration of alkali increased citrate excretion as it did in This was greatly favored by substitution of sucrose for casein in diet, but the addition of sucrose without lowering the casein did produce this result. They conclude that since animals excrete so n citrate when fed citrate-low diets during prolonged alkalosis, since there are no stores of citric acid in blood, liver, muscle, or ney, the body is capable of synthesis of citric acid.

Orten and Smith ('37) injected into dogs the disodium salt malonic, succinic, fumaric, malic, and maleic acids while the ani were maintained on a constant, low citrate, basal diet. All of t compounds markedly increased the citrate excretion, although produced only moderate increases in the urinary pH. Injection sodium salts of acetic, glycolic, glycinic, lactic, and gluconic acids not significantly increase citrate elimination. It appears, there that the dicarboxylic acids named, or their metabolites, contribut the synthesis of citric acid in the organism.

Oxalic Acid.—Oxalic acid is also very widely distributed in f of vegetable origin. Small amounts are found in potatoes, beans, b tomatoes, cauliflower, onions, mushrooms, celery, currants, raspber grapes, pears, many other fruits and vegetables, tea, coffee, and co This acid is especially abundant in rhubarb and spinach among vegetables ordinarily eaten. During the World War there were ported some cases of oxalic acid poisoning in England as the re of eating rhubarb leaves, which contain more oxalic acid than do leaf stalks that are usually eaten. Children have been poisoned eating sorrel, whose pleasant acid taste is due to its high conten

his acid. The ordinary "acid" fruits such as apples, oranges, lemons, matoes, pineapples, and strawberries, contain very little oxalic acid, heir acids being mostly malic and citric.

Oxalic acid is very difficult to oxidize in the body; most of that hich is ingested is excreted. The ingestion of oxalic acid or its salts nds to prevent absorption of calcium from the alimentary tract, nce calcium oxalate is very insoluble under the conditions prevailing the body. Calcium oxalate sometimes constitutes the calculi of renal uretal origin. Götting ('09) cites changes in the bones indicating calcification as a result of feeding oxalic acid. Fincke and Sherman '35) found decreased utilization of calcium by growing rats fed a iet one-half of which was derived from spinach. Kohman and Sanorn ('35) found that feeding soluble oxalates caused excessive calcium xcretion in the urine. On the other hand, Mackenzie and McCollum '37) found that on a diet of purified foodstuffs containing optimal mounts of calcium, phosphorus, and vitamin D, and otherwise satisctory, the presence in the diet of 0.9 per cent of potassium oxalate id not affect growth or the per cent of bone ash in the rat in a period 10 weeks. Feeding 2.5 per cent of potassium oxalate did not affect rowth, but did lower the per cent of ash in the bones. The presence the diet of 0.06 to 2.5 per cent of potassium oxalate did not grossly fluence calcium excretion. On the same type of diet, but deficient in tamin D, the presence of 1.7 per cent of the oxalate hindered rowth and bone formation. It was found that even when oxalate nd calcium were fed in amounts of equal molarity, some of the lcium was absorbed and utilized, as shown by growth, per cent of one ash, and calcium excretion.

There are many reported observations on the excretion of oxalic id and oxalates in various diseases, but the results are not sufficiently nclusive to warrant discussion here. It is obvious that the presence oxalic acid or soluble oxalates is undesirable beyond the content in hich they are found in foods which provide but small amounts.

Aromatic Organic Acids in Foods.—Benzoic acid is present in anberries and prunes to a greater extent than in any other common ods. Whereas malic and citric acids are easily oxidized in the body carbon dioxide and water, and the sodium or potassium ingested as lts of these acids remain in the body fluids in the form of their carbonates, thus exerting an alkalizing action, benzoic acid is not pable of destruction in the body. It is mildly toxic and the body njugates it with the amino acid glycine to form hippuric acid, which excreted by the kidneys. The amounts of benzoic acid in these uits is so small, however, that it is questionable whether it has any actical significance in nutrition, since these fruits are ordinarily ten in relatively small amounts. These foods do, however, have the

property of contributing to the acidity in the acid-base balance. S the glycine necessary for the formation of hippuric acid is synthes by the body, the loss of this amino acid is of little or no importanc

Quinic Acid, or hexahydro-tetrahydroxy benzoic acid, has shown by Kohman and Sanborn ('31) to occur in prunes and c berries along with benzoic acid, the amount of quinic acid pre being about 1 per cent. Quinic acid, when ingested, is converted benzoic acid and is then transformed into hippuric acid.

Salicylic Acid.—Salicylic acid has about the same distributio malic and citric acids in that almost all fruits and berries, grapes, contain it in small amounts. Quick ('33) states that this acid is converted into benzoic acid and only a small amount is conjuga with glycine to form "salicyluric acid." Most of the salicylic a administered to human subjects seems to be excreted as the free a

Lactic Acid.—Lactic acid is an intermediary in carbohydr metabolism and occurs in considerable amounts in the work muscle. It is ordinarily ingested in appreciable amounts when s milk is taken or when lactose is eaten; lactic acid may also a through fermentation of lactose in the alimentary tract. It has alrea been noted that the increased acidity in the intestine produced feeding lactose facilitates the absorption of calcium. It is well demo strated that when absorbed into the blood, lactic acid is capable being converted into glycogen. It may, therefore, replace to a certa extent carbohydrate in the diet. Lactic acid is easily dealt with by t body in useful ways. Its formation in the colon, when lactose is f liberally, may in most subjects bring about a transformation of t bacterial flora from the protein-decomposing types to a fermentati type, with consequent reduction of the extent to which unwholeson products of protein putrefaction are formed in the colon. There h been much discussion as to whether the latter products are of phy ological significance. Certainly they are unwholesome, have physiological value, and pollute the blood stream.

Succinic Acid.—This acid occurs in muscle. It is found in ma vegetable foods in slight amounts. Annau and coworkers ('35) ha proposed a scheme representing an essential catalytic system in t respiration of tissues, involving Warburg's respiratory enzyn (riboflavin-protein complex), cytochrome, and an unknown inte mediate. According to their scheme, oxygen uptake is accomplish by fumarate which is oxidized to oxalo-acetate; the latter molecu oxidizes the activated hydrogen donators and undergoes reduction succinate and to fumarate. Succinate is oxidized to fumarate whi reestablishes the catalytic cycle. This hypothesis, which is supported considerable experimental evidence, is interesting in that it attribut to certain four-carbon acids a role in intermediary metabolism.

The Effects of Vitamin Deficiency on Carbohydrate Metabolism. —Sure and Smith ('31) observed that in vitamin A deficiency so pronounced as to cause severe eye lesions, there is no significant change in the concentration of "true" blood sugar as compared with normal animals. The "apparent" sugar of the blood in the experimental animals was frequently considerably higher in the pathological animals than in the controls. They also report that in rickctic rats the blood sugar, alkaline reserve, and liver glycogen values are within the normal range. Similar results were obtained using vitamin G-deficient rats. Shimada ('34) found no differences in the carbohydrate tolerance and no disturbance of carbohydrate metabolism in scorbutic as compared with normal guinea pigs. On the other hand, Sigal and King ('36) found that successive stages of depletion of ascorbic acid (10, 15, and 20 days) induce a corresponding rise in fasting blood sugar level and a distinctly lowered glucose tolerance. The typical peak in the blood sugar curves moves characteristically upward and to the right with successive stages of ascorbic acid depletion. Administration of 10 mg. of ascorbic acid daily after 20 days' depletion induces a return to normal within 15 days.

The Effects of Inorganic Elements on Sugar Metabolism.— McQuarrie, Thompson and Anderson ('35), having observed that certain diabetic patients exhibited an abnormal craving for common salt, made observations which led to the discovery that the ingestion of between 1 and 2 gm. daily of sodium chloride per kilogram of body weight exerted a favorable influence on the carbohydrate metabolism of diabetic children taking simplified diets low in potassium. The effect was usually observable on the second or third day. High salt consumption caused significant elevation of both systolic and diastolic blood pressure levels. One of their patients required 60 to 90 gm. of sodium chloride daily to satisfy his craving for salt. Sodium appeared to be chiefly responsible for these effects, since other salts of this element likewise exerted these effects, but to less marked extent. Potassium chloride had diametrically opposite effects on both glycosuria and blood pressure. In terms of chemical equivalents, potassium completely antagonized the effects of sodium when given simultaneously in amounts as little as one-third that of sodium. The ingestion of sodium chloride caused a marked reduction in the degree of glycosuria. The fasting blood sugar was found to range at lower levels after a few days of high sodium chloride ingestion than during the foreperiod. In severe diabetes, ketonuria appeared earlier after withdrawal of insulin when the salt intake was low than when it was high. Ingestion of much salt changed the nitrogen balance from negative to positive.

Crabtree and Longwell ('36) carried out experiments, suggested

by the findings of Chaikelis ('34) that there is an inverse r between blood chlorides and blood sugar, to determine whether amount of sodium chloride in the diet would exert an influence u the deposition of glycogen in the liver and muscles of the rat. T found that a high salt diet almost doubled the glycogen content the livers as compared with the content in the control and the sodium chloride groups. Muscle glycogen seemed not to be affec This phenomenon has not been further investigated.

Blatherwick, Bell and Hill ('24) observed no consistent change the blood phosphate of humans upon oral administration of dextro Speirs and Sherman ('36) state that calcium and phosphorus utili tion was not influenced by the inclusion of dextrose, dextrin, co starch, or sucrose, respectively, in liberal amounts in the diet.

Keil and Nelson ('34) found in a study of the effects of copp on anemic rats that the oral administration of this element alo produces a different type of glucose tolerance curve than that obtain in the same animal before the mineral ingestion. A significant lowe ing of the maximum point demonstrates a role of copper in nutritio aside from its hemopoietic function. An increase in hemoglobin anemic rats produces a proportional increase in sugar toleranc coupled with a lowered glucose level in the blood after a 24 hour fas In their experiments there was no change in the hemoglobin of th blood, which led them to attribute the effects of copper to som process other than ordinary oxidative processes. Pure iron alone di not improve glucose utilization in their anemic rats as did copper.

Nutritive Requirements.—Carbohydrate is the most easily avail able and most economical source of energy for the animal body. A certain minimum amount is essential for the metabolism of othe foods, especially fats, to prevent the development of ketosis. A reason able excess above this minimum is desirable in order to meet a portion of the requirements for energy without necessitating the intake o exceedingly large amounts of fat and proteins.

The nutritional requirement for carbohydrates varies according t the activity of the individual. It is believed that the carbohydrate furnish about 60 per cent of the fuel supply of the average norma adult. The minimum amount of carbohydrate necessary for mainte nance of life and the prevention of ketosis is from 10 to 15 per cen of the total energy requirement.

REFERENCES

Cori and Cori (1928). J. Biol. Chem., *76*, 755.
Cori (1926). Proc. Soc., Exper. Biol. & Med., *23*, 290.
Groen (1937). J. Clin. Investig., *16*, 245.

Goldblatt and Ellis (1932). Biochem. J., *26*, 991.
Dann and Chambers (1930). J. Biol. Chem., *89*, 675.
Dann and Chambers (1932). J. Biol. Chem., *95*, 413.
Deuel, MacKay, Jewel, Gulick and Grunewald (1933). J. Biol. Chem., *101*, 301.
Bell (1935). Biochem. J., *29*, 2031.
Bell (1936). Biochem. J., *30*, 2144.
Haworth and Percival (1932). J. Chem. Soc. 2277.
Young (1936). J. Physiol., *87*, 111.
Bell and Young (1934). Biochem. J., *28*, 882.
Deuel, MacKay and Gulick (1932). Proc. Soc. Exper. Biol. & Med., *30*, 24.
MacKay and Bergmann (1933). J. Nutr., *6*, 515.
Murlin, Nasset, Murlin and Manley (1936). J. Nutr., *12*, 645.
Deuel, Gulick and Butts (1932). J. Biol. Chem., *98*, 333.
Goldblatt (1925). Biochem. J., *19*, 948.
Wesson (1927). J. Biol. Chem., *73*, 507.
Wesson and Murrell (1933). J. Biol. Chem., *102*, 303.
Wesson and Murrell (1934). J. Biol. Chem., *105*, xcix.
Miller and Lewis (1932). J. Biol. Chem., *98*, 133, 141.
Russell (1936). Proc. Soc. Exper. Biol. & Med., *34*, 279.
Fisher and Pencharz (1936). Proc. Soc. Exper. Biol. & Med., *34*, 106.
Russell and Bennett (1936). Proc. Soc. Exper. Biol. & Med., *34*, 406.
Fisher, Russell and Cori (1936). J. Biol. Chem., *115*, 627.
Evans (1936). Am. J. Physiol., *114*, 297.
Buell, Anderson and Strauss (1936). Am. J. Physiol., *116*, 274.
Butts, Dunn and Hallman (1935-1936). J. Biol. Chem., *112*, 263.
Samuels, Butts, Schott and Ball (1936-1937). Proc. Soc. Exper. Biol. & Med., *35*, 538.
Barnes and Regen (1934). Endocrinology, *17*, 522.
Deuel and Gulick (1932). J. Biol. Chem., *96*, 25.
Deuel, Gulick, Grunewald and Cutler (1933). J. Biol. Chem., *104*, 519.
Greisheimer (1931). J. Nutr., *4*, 411.
Stohr (1932). Ztschr. f. physiol. Chem., *212*, 121.
Gulick, Samuels and Deuel (1934). J. Biol. Chem., *105*, 29.
Gayet and Guillaumie (1927). Compt. rend. Soc. de biol., *97*, 1613.
Gayet and Guillaumie (1933). Compt. rend. Soc. de biol., *112*, 1194, 1327.
Kosaka (1933). J. Physiol., *79*, 416.
Lusk (1915). J. Biol. Chem., *20*, 555.
Deuel and Chambers (1925). J. Biol. Chem., *65*, 7.
Greisheimer and Johnson (1930). Proc. Soc. Exper. Biol. & Med., *27*, 769.
Mitchell (1927). Am. J. Physiol., *79*, 542.
Koehler and Allen (1934). J. Nutr., *8*, 377.
Whittier, Cary and Ellis (1934). J. Nutr., *9*, 521.
Jarvis (1930). Am. J. Dis. Child., *40*, 993.
Feyder (1935). J. Nutr., *9*, 457.
Mitchell (1937). J. Nutr., *14*, 435.

Dragstedt and Peacock (1923). Am. J. Physiol., *64,* 424.
Hudson and Parr (1924). J. Infec. Dis., *34,* 621.
Kline, Keenan, Elvehjem and Hart (1932). J. Biol. Chem., *98,* 1
Inouye (1924). Am. J. Physiol., *70,* 524.
Barenberg and Abramson (1930). Arch. Pediat., *47,* 1.
Gerstley (1930). J. Am. Med. Assoc., *95,* 1233.
Escherich (1886). "Darm Bacterien der Sauglings." F. Enke, Stutt
Kopelloff and Cohen (1930). J. Am. Med. Assoc., *94,* 1983.
Watkins (1928). J. Biol. Chem., *80,* 33.
Cori (1925). J. Biol. Chem., *66,* 691.
Mathews (1925). "Physiological Chemistry." 4th Ed. Wm. Wood & C
New York.
Mitchell and Dodge (1935). J. Nutr., *9,* 37.
Day (1936). J. Nutr., *12,* 395.
Mitchell (1936). J. Nutr., *12,* 447.
Yudkin and Arnold (1935). Proc. Soc. Exper. Biol. & Med., *32,* 836
Mitchell, Cook and Merriam (1937). J. Nutr., *13,* 18.
Fridericia (1926). Report on Refection at XII Int. Congress on Physiolo
Stockholm. Skand. Arch., *49.*
Roscoe (1927). J. Hygiene, *27,* 103.
Bliss (1935). J. Nutr., *11,* 1.
Guerrant, Dutcher and Tomey (1935). J. Biol. Chem., *110,* 233.
Guerrant and Brown (1937). J. Nutr., *13,* 305.
Kelly and Parsons (1937). J. Nutr., *13,* 453.
Blatherwick and Long (1922). J. Biol. Chem., *53,* 103.
Sherman, Mendel and Smith (1936). J. Biol. Chem., *113,* 247.
Ostberg (1931). Skand. Arch. Physiol., *62,* 81.
Orten and Smith (1937). J. Biol. Chem., *117,* 555.
Götting (1909). Virchow's Arch. f. path. Anat. u. Physiol., *197,* 1.
Fincke and Sherman (1935). J. Biol. Chem., *110,* 421.
Kohman and Sanborn (1935). Ind. & Eng. Chem., *27,* 732.
Mackenzie and McCollum (1937). Am. J. Hyg., *25,* 1.
Kohman and Sanborn (1931). J. Ind. & Eng. Chem., *23,* 126.
Quick (1933). J. Biol. Chem., *101,* 475.
Annau, Banga, Gozsy, Husack, Laki, Strab and Szent-Györgyi (1935)
Ztschr. f. physiol. Chem., *236,* 1.
Sure and Smith (1931). Proc. Soc. Exper. Biol. & Med., *28,* 349, 440, 44
Shimada (1934). J. Biochem., *19,* 237.
Sigal and King (1936). J. Biol. Chem., *116,* 489.
McQuarrie, Thompson and Anderson (1935). J. Nutr., *11,* 77.
Crabtree and Longwell (1936). Proc. Soc. Exper. Biol. & Med., *34,* 707
Chaikelis (1934). J. Biol. Chem., *105,* 767.
Blatherwick, Bell and Hill (1924). J. Biol. Chem., *61,* 241.
Speirs and Sherman (1936). J. Nutr., *11,* 211.
Keil and Nelson (1934). J. Biol. Chem., *106,* 343.

CHAPTER

IV

Lipids in Nutrition

BIOCHEMISTS have been increasingly attracted to researches relating o fats, fatty acids, glycolipids (compounds of fatty acids with carbo-ydrate, and containing nitrogen but not phosphoric acid), phospho-ipids, sterols, and hydrocarbons which occur in plant and animal ubstances. Much of this work deals with isolation and study of the hemical characteristics of lipids, their occurrence in different tissues, nd the variation of their content under different conditions. The tudent of nutrition is primarily interested in the nutritive significance f these substances, particularly as respects the capacity of the body o synthesize them, whereas the biochemist is concerned with elucidat-ng all the chemical transformations which these bodies undergo in netabolism. But the student of nutrition has a keen interest in learning hat effects disturbances of nutrition may bring about in the behavior f any and all chemical substances with which the body has to deal. His inquiries must, of necessity, lead him to consider all phases of athology which are influenced by the nutritional state. In this chapter more arbitrary selection of topics is made for consideration since pace does not permit of an extended discussion of all phases of pid metabolism.

Effect of Diets Deficient in Fat.—Several investigators have udied the effects of diets which contained no fats. The observations now that rats fail in health when deprived of all fats. Burr and urr ('29), using rats, first described the fat-deficiency syndrome. Owing to accumulation of dried secretion the eyelids tend to stick gether, and there is loss of hair beginning on the head which may ontinue to almost complete denudation. Dermatitis develops on the ars, neck and upper part of thorax, forearms, backs of forepaws, ains, and backs of hind paws. There is a yellowish incrustation on

the reddened and thickened ears, and frequently a linear fissuri ulceration at the angles of the mouth, and a lesion at the tip tongue, which first appears as a small, roughly circular grayish o or bleb or as an ulcer, which in some cases forms a yellowish sl A striking symptom is necrosis of the tail.

This dietary deficiency disease is entirely distinct from what formerly called "rat pellagra," but which is now known to have misnamed, and is usually designated "rat dermatitis." It woul better named nutritional dermatosis, since it appears that chang the skin involve factors other than inflammation. The rat derma is produced by diets deficient in vitamin B_6 but containing such as cod liver oil and Crisco, either of which would prevent fat-deficiency syndrome.

Fat-free diets regularly produce kidney lesions in the rat, kidney degeneration probably causes the death of the animals most cases. High protein feeding seems to increase the severity of kidney damage, since it is accompanied by hematuria. A cur phenomenon is that the small emaciated rats on the fat-free d drink twice as much water as their controls on the same diet c taining fat. Ovulation is irregular or ceases entirely in these anim but is resumed on feeding them the essential fatty acids. When fem rats on the diet do ovulate they mate and produce litters but these inferior young, probably because of the poor nutritive condition the mother. Male rats on the fat-free diet will generally not ma while their controls receiving 10 per cent of lard, mate and sire norm litters. Fat-free diet males which do mate cannot sire young. T new type of sterility differs from that induced in males made ste by vitamin E deficiency in that the fat-deficient animals quickly l their sex response, while those lacking vitamin E retain it even af degeneration of the seminiferous epithelium.

When young rats are restricted to a fat-free diet, otherwise a quate, growth is not much retarded during the first 60 days, but af this time the rate of increase in weight falls off and they plateau weights of 160-180 gm., which is about 100 gm. less than the contr on the same diet plus fat. A group of rats under these conditions by the time they have reached the age of 240 days. Rats getting t diet with fat during the same interval are much larger and rem in good health.

Indispensable Unsaturated Fatty Acids.—This nutritional dise is curable by the inclusion of 2 per cent of fatty acids from cert sources. Glycerol and the non-saponifiable fraction of fats are ineff tive. Storage fat can be almost entirely used up when rats are restric to a fat-free diet. Burr and Burr suggested that the amount of bo fat can be controlled over a wide range by the addition of min

quantities of fat to the diet. After extensive investigations Burr and associates ('32) discovered that both linoleic and linolenic acids are effective in preventing or curing the fat-deficiency disease. These fatty acids seem to be of about equal value and can replace each other in the tissues. Contrary to the earlier findings of Burr and coworkers, Turpeinen ('38) found that arachidonic acid, $C_{20}H_{32}O_2$, which contains 4 double bonds, is superior to linoleic or linolenic acid as a growth restorative. He explains the apparently conflicting results in his studies and those of Spadola and Ellis ('36), who reported that the rat is able to synthesize arachidonic acid, by suggesting that this synthesis can be accomplished only from, or in the presence of, linoleic or linolenic acid. The need of the animal may be primarily for arachidonic acid, and linoleic or linolenic acid may be of value because of the capacity of the body to convert them into arachidonic acid. Oleic acid, stearic, palmitic, myristic, lauric, and lower fatty acids are ineffective, and alpha eleostearic acid, an isomer of linolenic acid, is of no value in preventing the disease. Also, as shown by Turpeinen (l.c.), erucic, ricinoleic, $\Delta^{12:13}$-oleic, and chaulmoogric acids are ineffective. Butter fat and tung oil contain little of the curative acids. Olive oil, lard, corn oil, linseed oil, egg yolk fats or lecithin from egg yolk, and poppy seed oil contain relatively large amounts of linoleic or linolenic acid or both, and are curative.

From these studies it is apparent that the rat, and presumably other mammals, require linoleic acid, linolenic acid, or arachidonic acid as nutrients. It is, of course, well known that the animal body is capable of synthesizing from carbohydrates, and from those amino acids which are glucose formers, all of the fatty acids which are commonly found in the body fats of animals. The body does not, however, possess the power to synthesize the unsaturated fatty acids of the linoleic or linolenic acid type and arachidonic acid. These are indispensable for some metabolic processes, or as structural units of protoplasm.

Brown and Burr ('36) state that it is not necessary to use highly purified food substances in order to produce the fat-deficiency syndrome. Crude casein, when substituted for purified casein, raises the weight of rats slightly at the time of cessation of growth. Decline and death are postponed, but hematuria, scaliness, and tail necrosis occur to almost the same extent. Substitution of fresh skim milk for casein and potato starch for part of the sucrose lead to results similar to those obtained with crude casein. Yeast oil and buttermilk lipids, high in unsaturated fatty acids, are non-curative.

Sinclair ('36) points out that although the growth of rats on a diet devoid of triply unsaturated fats is distinctly subnormal, it is nevertheless very considerable. The source of the essential unsaturated acids

which must have been available has remained unsettled. He fed upon a diet of casein, salt mixture, yeast, and elaidin (elaidic ac the trans-form corresponding to oleic acid which is the cis-fo supplemented with cod liver oil concentrate. The elaidin supplie per cent of the calories. After 3 to 4 weeks, when the ani weighed 80 to 90 gms., growth ceased, and their weights rema constant for some weeks until decline set in, terminating in deat the age of about 4 months. When growth had been arrested for a weeks, some animals were fed a diet in which sucrose replaced elaidin. Immediately growth was resumed at the rate of about 20 a week, the animals eventually reaching weights comparable to th of rats fed from weaning age on the same fat-free diet. Elai therefore, caused cessation of growth earlier than would have occu on a fat-free food. Replacement of 5 per cent of the elaidin b mixture of cod liver oil and corn oil in equal parts resulted excellent growth, showing that the elaidin was not toxic. Sinc suggests that the high intake of elaidin—and presumably of ot fats devoid of the essential unsaturated acids—almost comple abolishes the limited synthesis of linoleic or linolenic acid fr carbohydrate, and thus prevents growth. His experiments suggest t the body is not without the power to synthesize these acids, but t they cannot be synthesized fast enough to meet the requirements the animal for growth.

Sinclair ('32) found that the degree of unsaturation of the ph pholipid fatty acids in the entire body of the rat, as shown by t iodine number, was about 102 for those fed a fat-free diet, a increased to 125 when 50 mg. of cod liver oil was fed daily. T value increased progressively to a maximum value of 140 when 1 150, 200, and 250 mg. were fed daily. The corresponding figures f saturated fatty acids ranged from 63 to 72 approximately. The effect feeding small amounts of cod liver oil on the degree of unsaturati of the fatty acids of the phospholipids was much greater in the liv phospholipids than in those of the skeletal muscles. Lard and cocon oil, when added to the fat-free basal ration, produced similar effec Feeding cod liver oil with the fat-free basal diet does not prevent t development of the typical lesions caused by fat deficiency. It is al shown that these lesions do not develop when rats are kept in ordina stock cages, but only when kept in false bottom cages which preve access to the animals' feces.

Sinclair points out that since the maximum iodine value phospholipids derived from oleic acid, which the rat can certain synthesize, is 90, whereas that of animals fed the fat-free diet is 1 or over, the animals must have synthesized some more highly u saturated fatty acids while on the latter diet. The fact that on t

fat-free diet they do grow, although more slowly than normal, points to this possibility.

The Metabolic Rate and Respiratory Quotients of Rats on a Fat-Deficient Diet.—Wesson and Burr ('31) subjected rats for some time to a fat-deficient diet and determined their metabolic rates and respiratory quotients. During the first hours after feeding carbohydrate to such rats the respiratory quotients were well above unity, which indicates that they were forming fat from the absorbed carbohydrate. The fat thus synthesized does not relieve the animals from the symptoms of their deficiency disease, because linoleic or linolenic acid are not formed, or, as seems plausible from the studies of Sinclair, are not synthesized fast enough to meet the nutritive requirements for them. The formation of fat from carbohydrate is a normal process of well fed animals whose glycogen stores are practically filled. After the stores of glycogen are depleted by fasting, fat synthesis would, on the basis of present knowledge, cease because of lack of carbohydrate reserves and because protein of the tissues in the normal individual is never drawn upon as a source of energy (part of which comes from carbohydrate synthesized from amino acids) beyond the actual immediate needs. It is a matter of common observation that fasted animals lose fat, and under conditions of energy deficit it has been assumed that fat formation is at a standstill.

The high R.Q. (respiratory quotient) of the emaciated animals, as they become progressively free from fat notwithstanding a generous intake of food, is remarkable. The administration of linoleic or linolenic acid causes an immediate increase in weight and an excessive gain in fat, which is shown by the R.Q. to be derived, at least in part, from carbohydrate.

Wesson ('27) fed rats a restricted diet consisting of dextrin, traces of iodine, calcium carbonate, sodium chloride, and small amounts of rolled oats. Rats taking this diet during a two weeks period had R.Q.s as high as 2, which indicates rapid formation of fat during the assimilation of a carbohydrate meal. When this diet was modified by the inclusion of casein high R.Q.s were still observed. If a small amount of lard, liver, or kidney fats was fed (0.4 to 1.0 gm.) the animals showed a day or two later R.Q.s of 1.0 or lower, i.e., normal assimilative respiratory quotients were obtained.

Wesson and Burr (l.c.) have studied further this interesting behavior of rats suffering from fat deficiency. The remarkable fact is that, whereas these rats can synthesize considerable amounts of fat under the experimental conditions, it does not relieve them of their symptoms or correct their aberrent metabolism; but the administration of very small amounts of linoleic or linolenic acid (in lard or other fats) at once changes the manner in which the body deals with

carbohydrate which is being assimilated. The basal and assimil metabolic rate in the case of rats showing early symptoms of fat-deficiency disease was well above the normal value, while metabolic rate in the later stages of the disease was norma subnormal. The temperature of the rats in fat-deficiency disease normal. Miyazaki and Abelin ('24) and Abelin ('26) reported normally high specific dynamic action of carbohydrate in patients fering from hyperthyroidism and were able to reduce it by fat feed Wesson and Burr could not accomplish a reduction of the metab rate in their rats by this means. The cause of the high metabolic in their animals remains unexplained.

Finally, Wesson and Murrell ('34) have demonstrated that partial hydrogenation of the alcohol-soluble fraction of lard, lead to the complete or almost complete conversion of the linoleic a which it contains into a saturated fatty acid, does not diminish effectiveness of the fat with respect to lowering the abnormal respi tory quotients of rats with fat-deficiency disease. They are, therefo led to conclude that the factor influencing carbohydrate metabolism not linoleic acid. The latter is, however, definitely preventive curative for the fat-deficiency disease. The carbohydrate factor is s of unknown nature.

Effect of Fat-Deficiency on Humans.—Since a predomina symptom of fat-deficiency in rats is a scaliness of the skin, t possibility has been investigated that certain eczematous conditions humans, particularly of infants, might be related to a deficiency essential fatty acids, or a disturbance in their metabolism (Hanse '33; Epstein and Glick, '37). As shown by the important studies Hansen and his associates, the blood of eczematous infants tends have less unsaturated fatty acids than normal. The liberal administr tion of fats yielding high amounts of unsaturated fatty acids raise the degree of unsaturation of blood fats and, in a gratifying degre alleviated the eczema. Further investigation should clarify the relatio of dietary fat to eczematous conditions, but at present there is sca basis for the extravagant claims, frequently seen, that the indispensab fatty acids should be rubbed into the skin as an aid to beaut Exploiters of these nutrients refer to them as "vitamin F" or "sk vitamin." Obviously the purpose is to promote sales since designatio of a nutrient as a vitamin increases its sales-appeal.

A normal adult man subsisting 6 months on a diet extremely lo in fat experienced no evidence of disease but the serum fatty aci became more saturated and the content of serum linoleic and arach donic acids decreased approximately 30 per cent (Brown, Hanse Burr and McQuarrie, '38). The R.Q. rose to 1.14 following a me of the diet, indicating fat synthesis. The evidence from this stu

suggests that humans do require fatty acids in the diet, but further investigations are needed before definite conclusions can be drawn.

The Effect of Fats on Galactose and Lactose Utilization.—Schantz, Elvehjem and Hart ('38) have made important observations on the effect of fats in the diet on the utilization of milk sugar and galactose. Rats fed whole milk, supplemented with iron, copper, and manganese (in which milk is deficient) were very efficient in the utilization of the milk fats. A pig and a calf were studied with the same result. When, however, the animals were confined to a diet of skim milk, similarly fortified with minerals, galactose was excreted in the urine after a few days' feeding. As much as 35 per cent of the galactose of the milk sugar was recovered in the urine. When butter fat, lard, corn oil, coconut oil, palmitic and oleic acids were added to the diet to the extent of 3 to 4 per cent the loss of galactose in the urine was prevented. Glycerine, butyric, beta-hydroxybutyric, caproic, and lactic acids did not prevent the loss. The blood sugar in rats fed the mineralized whole milk was about 140 mg. per cent. Whereas that of rats fed mineralized skim milk rose to about 200 mg. per cent.

These experiments show that some relation exists between the utilization of the galactose part of the molecule of milk sugar and the presence of certain fatty acids. The glucose portion of the sugar is utilized readily without fat. Schantz and associates found that rats fed fat-free diets containing glucose did not excrete sugar as they do on a lactose—or galactose—containing diet. They suspected that the presence of fat in the intestine might serve to delay absorption of galactose and thus prevent accumulation of this sugar in the blood and tissues beyond the powers of the body to catabolize it. However, they found that the feeding of skim milk at the rate of 2 cc. per half-hour did not prevent an abnormal rise in blood sugar and excretion of galactose for a few days.

These observations have a special interest in connection with the findings of Deuel, Gulick and Butts ('32), and of Clark and Murlin ('36) who state that galactose is superior to other hexoses in ketolytic activity. Deuel et al. ('36) have pointed out that fatty acids containing eight or more carbon atoms are much greater producers of beta-hydroxybutyric acid and acetoacetic acid formers than are butyric acid and caproic acid which the Wisconsin workers find to be ineffective in increasing the utilization of galactose.

The Role of Fat in the Phenomenon of Refection.—Whipple and Church ('35) found thiamin in the feces of rats on a thiamin-deficient diet containing 10 per cent of lard with sucrose as the only source of carbohydrate. When the diet was made fat-free the feces were free from thiamin. But little of the vitamin was formed when corn starch was substituted for sucrose. The symptoms of thiamin-

deficiency (polyneuritis) were produced more quickly on f diets than when fat was provided, and the larger the amount fed the longer were the symptoms delayed. The feeding alleviated the symptoms of polyneuritis in rats permitted to d the disease on a fat-free diet, but they eventually succumbed avitaminosis. The amount of thiamin eliminated in the fe directly correlated with the content of fat in the diet, which to be essential for the formation of the vitamin in the alim tract through the agency of yeast. This suggests that fat thiamin synthesis, probably by bacteria, in the lower intestine.

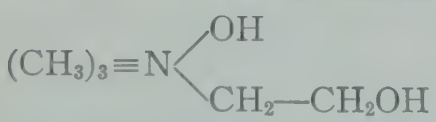
The observation of Whipple and Church is of interest in co tion with the studies of Evans and Lepkovsky ('29) from wh was concluded that fats exert an important function in the metab of the animal other than supplying energy. Fats, they found, w delay the onset of the symptoms of thiamin-deficiency when vitamin was fed in inadequate amounts. They raised the que whether feeding fats influenced in some manner the phenomen refection. Evans and associates ('34) have recently concluded tha fat to exert its optimal sparing action upon thiamin both protein the heat-stable factor (their vitamin G), must be high. The spa action of fats on thiamin requirements will be discussed in Cha XIX.

The Phospholipids.—In animals three types of phospholipids found: 1, the lecithins—substances consisting of two fatty a glycerol, phosphoric acid, and the nitrogenous base, choline; 2, cephalins—substances similar to the lecithins except for the subs tion of aminoethyl alcohol for choline; 3, the sphingomyelins— stances related to lecithins in that they contain phosphoric acid choline, and to the cerebrosides in that they contain the nitroge base, sphingosine, and, in part, C_{24} fatty acids. Since these lipids all capable of synthesis in the animal body they have been gene believed to be dispensable in the diet. They are extremely impor as structural elements in most tissues, but a discussion of these would be out of place here. The observations of Best and Channon their coworkers on the effects of administration of choline to ani have a nutritional bearing, however, and will be briefly considered

Hershey and Soskin ('31) investigated the cause for the l known phenomenon that depancreatized dogs suffer impairmen assimilation of protein and fat, and since the condition is gre ameliorated by feeding the dogs raw pancreas, the belief long preva that this benefit was due to the provision of the pancreatic diges enzymes by this means. Various investigators have reported depancreatized dogs did not survive more than 1 to 8 months o meat and sugar diet, notwithstanding complete control of diab

manifestations by the use of insulin, unless raw pancreas was also provided. This was interpreted to mean that the external digestive secretions of the pancreas were essential in the alimentary tract for the survival of such animals. Hershey and Soskin found that depancreatized dogs could survive for a very long time, presumably indefinitely, on lean beef muscle, cane sugar, and insulin, without pancreatic enzymes being given by mouth, provided they were given frequent doses of egg yolk "lecithin." Lecithin is put into quotation marks because the preparations used were never pure chemical entities, but crude material containing various impurities. Without "lecithin" the animals fail because of impairment of liver function characterized by fatty changes in the liver. When depancreatized dogs develop the characteristic syndrome, the addition of "lecithin" promptly alleviates the signs and symptoms of this condition. Depancreatized animals fed beef muscle and sucrose, with insulin, accumulate much fat in their livers. The administration of lecithin to these animals greatly increases their excretion of sugar in the urine.

Best and Huntsman ('32) studied the components of the lecithin preparation in order to identify the substance to which this material owed its remarkable effect. Oleic acid, glycerophosphate, and aminoethyl alcohol, proved ineffective, but choline,



possessed the same protective action as raw pancreas or lecithin in improving the well-being of the animals, and in preventing the liver damage associated with the accumulation of fat.

That one function of the liver is desaturation of fatty acids is clearly shown by experiments of Best, Hershey and Huntsman ('32) who fed rats their stock diet with added fat equivalent to 40 per cent of the food. The average fatty acid content of the livers in a group of 9 animals after 22 days on the diet was 18.3 per cent. The livers of a group of rats similarly fed, but with the provision of lecithin contained 5 per cent. The iodine number of the food fat was approximately 40. In rats not receiving lecithin the iodine number of the fatty acids of the livers was approximately 100, whereas that of the fatty acids of livers from a comparable group receiving lecithin was approximately 72. The minimum effective dose of choline is less than 3 mg. per rat per day but the amount necessary to maintain livers at their normal lipid content was 190 mg. per day. Choline not only prevents accumulation of fats in the liver but of cholesterol esters as well. The effect on the latter was less than on fats, being only about 60 per cent,

but these results were obtained with diets containing 2 per cen cholesterol.

It appears from studies of Best and of Channon ('35) that action of choline, as revealed in these experiments, is not conf exclusively to this substance. Casein which was sufficiently purifie to contain entirely insufficient amounts of choline to produce effects observed, had a choline-like action. It is estimated that 2 of highly purified casein are equivalent to 1 mg. of choline. It se necessary to conclude, provisionally, that some amino acid deri from casein, or perhaps a lipotropic factor such as betaine, is m available in the metabolism of the amino acids of casein. Gelatin been shown to possess no choline-like action. That the lipotrop action of choline is not specific is shown by the observations Channon and Smith ('36) that the triethyl homologue of choline effective in preventing deposition of fat in the liver, although action is not so intense as that of choline.

The choline content of various substances of biological interest h been determined by Fletcher and associates ('35). Their data a assembled in the following table:

TOTAL CHOLINE CONTENT OF ANIMAL TISSUE

Tissue	*Average Choline Content mg./100 g.*
Rat:	
Spermatic fluid	514
Spinal cord	370
Brain	325
Adrenals	304
Cerebellum	296
Cerebral hemispheres	274
Liver	260
Pancreas	232
Pituitary	224
Kidneys	202
Thyroid	167
Lungs	164
Heart	158
Lymph glands	152
Stomach	152
Spleen	151
Small intestine	142
Salivary glands	131
Tongue	123
Thymus	113
Skeletal muscle	100
Uterus	74
Skin	64
Bone	44
Connective tissue	40
Fat	23
Blood-starved	22
Blood-fed	31
Ox:	
Liver	270
Pituitary, anterior lobe	259
Pituitary, posterior lobe	217
Pancreas	230
Muscle	76
Blood, defibrinated	13
Fat	0.5–2.6
Dog:	
Liver	230
Stomach	90
Blood, whole	34
Pig:	
Pancreas	280
Bacon (cured side)	44
Fat (from cooking bacon)	6
Lard	1
Codfish:	
Muscle	78

These studies on the physiological role of choline are of great interest since they indicate that choline is an essential dietary factor when certain sources of protein supply the amino acid requirements of the body. It appears that choline, or a substance resembling it chemically and physiologically, may arise through synthesis from certain amino acids. The pathological manifestations of animals on a choline-free diet is much more easily demonstrated when the experimental diet is poor in protein, than when the protein intake is liberal. With moderately high protein diets these manifestations may not occur. It seems clear that the phospholipids as a source of choline contribute an essential dietary factor. A point of interest is that such cereal products as white flour, skimmed milk powder, rice flour from polished rice, and possibly other refined cereal flours, which are very deficient in fats and other lipids, are, nevertheless, relatively rich sources of choline or other substance exerting a choline-like action.

The Lipids in Living Tissues.—The conception that lipids are essential components of protoplasm arose in the minds of the earlier investigators of fatty substances in brain and other tissues. This subject has in recent years interested workers anew, and since methods for identifying the individual component lipids have been greatly improved, some new and interesting facts have emerged relative to the role of these substances in the life processes.

Bloor and his associates ('30) have reviewed the older literature on this subject. Klem ('35) found that two rats after being starved to death, contained 1.54 and 1.58 per cent respectively of fatty acids. The value for starved mice was 2.0. Terroine and Trimbach ('34) have discussed the composition of the tissues in respect to lipids and hold that there is a constant content characteristic of each tissue which is not altered by extreme changes in the nutritional status of the animal.

Bloor and associates found the total lipid content, on the basis of dry weight of uterine mucosa, lung, kidney, and beef pancreas, to be approximately the same. There is a definite but not large increase in the percentage of lecithin in the uterine mucosa at the time of greatest elaboration of endometrium, which is accompanied by an increase in free cholesterol. The amount of cholesterol esters present in the endometrium is always very low. The percentage of phospholipid in the corpus luteum of the sow varies greatly with the activity of the tissue, being 2 to 3 times as high during the period of activity (previous to estrus and during pregnancy) as at the time of formation or after retrogression. Free cholesterol increases up to and during the period of active functioning but to a much lesser extent. Cholesterol esters vary inversely with the activity of the gland, a high content being characteristic of the degenerated organ. These results support

the hypothesis that the phospholipid content of this tissue is a func of its physiological activity. The relation of free cholesterol is clear, but appears to be similar to phospholipid.

Bloor and Snider ('34) found the following amounts of phosp lipid fatty acids and of cholesterol in animal tissues.

LIPID CONTENT OF MUSCLE OF WILD RABBIT
(in Per Cent of Dry Weight)

	PHOSPHOLIPID FATTY ACIDS		CHOLESTEROL	
	Average	Range	Average	Range
Heart	5.2	4.0–5.7	0.45	0.36–0.6
Jaw	3.2	2.6–3.8	0.36	0.23–0.3
Diaphragm	3.2	2.2–3.8	0.29	0.20–0.3
Neck	2.9	2.5–3.8	0.31	0.26–0.3
Front leg	3.1	2.6–4.2	0.27	0.18–0.3
Thigh	2.9	2.5–3.4	0.25	0.17–0.3
Loin	2.6	2.2–3.0	0.25	0.15–0.27
Stomach	2.6	2.5–2.8	0.50	0.45–0.65
Abdominal wall	2.5	1.6–3.4	0.24	0.12–0.40
Back	2.4	1.9–3.2	0.22	0.17–0.33
Forearm	2.36	1.8–2.7	0.27	0.08–0.28
Pectoralis	2.2	1.9–2.7	0.28	0.20–0.44
Gastrocnemius	2.1	1.9–2.6	0.22	0.15–0.28

LIPID CONTENT OF PECTORAL MUSCLES OF BIRDS
(in Per Cent of Dry Weight)

	PECTORALIS MAJOR		PECTORALIS MINOR	
	Phospholipid Fatty Acids	Cholesterol	Phospholipid Fatty Acids	Cholesterol
Pigeon	4.7	0.25	2.10	0.18
Owl	3.7	0.26	1.92	0.28
Rooster	1.37	0.17	1.10	0.18

These values indicate that the more used the muscle the higher i the content of phospholipid. Increased activity is accompanied b increase in phospholipid. The iodine number of the phospholipid fatt acids showed no difference in much used or little used muscles. Th differences in cholesterol content, when they occur, are in the sam direction, but not so great.

Sinclair ('34) in a review on the physiology of phospholipids, suggests that the functions of the phospholipids are (1) as possible oxygen-transporting substances due to the ability of their double bonded carbons to take up and probably to transfer oxygen; (2) as intermediaries in the metabolism of fats, as regards the early stages of fat absorption and transport, but doubtful in the later stages of combustion in the tissues; (3) as structural elements in tissues; and (4) in increasing transport fat in the cells owing to the solvent action on fat of the phospholipids.

In harmony with these views is the finding of Boyd ('35) that an inactive tissue, Wharton's jelly (gelatinous embryonic connective tissue of the umbilical cord), contains only about 1 per cent of phospholipids on the dry basis. The cholesterol ester content was insignificant, which is in accord with the general finding that a high content of these esters is characteristic of degenerating rather than active tissues. The generalization that phospholipids are abundant in highly active, and less so in inactive tissues, is further supported by analyses of tumors and of the mammary gland.

Boyd has reported interesting observations on the phospholipids and the associated variations in free and esterified sterols of the blood under different conditions. After surgical operation of any cause the phospholipids of the white blood corpuscles increase by 200 per cent if recovery sets in, and fall by a like amount if post-operative complications ensue. If recovery is normal after fever, the content of phospholipids in these cells increases, but falls if the illness proceeds fatally. There is a marked increase in phospholipids in the white cells of rabbit blood following parturition, the values returning to normal in three weeks. The phospholipid content of rabbit ovary increases from the beginning of pregnancy to 300 per cent of the normal value by the 15th day, and returns to normal just before parturition.

The Toxicity of Certain Fats and Fatty Acids.—It is a matter of common experience that cod liver oil, especially in the adult, may produce anorexia and gastric discomfort. This may well be attributable to its disagreeable taste and oily nature. Fats, when taken too liberally, inhibit gastric secretion and motility, but cod liver oil is more disturbing than most other fats. The effects may reasonably be attributed largely to its psychic influences, especially its flavor. Normal adults taking 2 ounces of cod liver oil daily have been reported to suffer from tachycardia and precordial discomfort. Agduhr ('26) found, however, that cod liver oil when given continuously to animals may produce toxic effects on the heart and the development of abnormalities such as pigment degeneration and fatty degeneration of the heart muscle, transformation of the muscle cells into connective tissue, and calcareous incrustations. Parallel experiments with olive oil, rape oil,

and coco fat, did not produce these changes. He did not includ his studies observations on other fish oils, which are, in gen characterized by a high degree of unsaturation. It seems probable Agduhr's results were magnified by the conditions of his experim especially by the use of a diet composed in great measure of pur food materials and consequently by the probability that cer nutrients were not present in the diet in sufficient amounts. In o words, it seems probable that the basal diet was not such as wo promote optimal nutrition.

This view is supported by the studies of Harris and Moore (who found that supplementing the diet with the vitamin B-comp inhibits the toxic effect of cod liver oil. It has been shown by Ha and Innes ('31) that vitamin D in excessive doses is much more to when added to diets composed of purified foodstuffs than when ad to diets of natural foods. The principle is further illustrated by protective effect of a high protein diet in offsetting the effects of l of choline and by the effects of amino acid imbalance. Experimen diets have been frequently shown to provide some one or mo essential nutrients in amounts below the optimal requirements whi bring animals into a condition of lowered tolerance for any deficien or excess of another constituent of the diet which imposes a physi logical burden upon the body. Most of Agduhr's experiments we made with calves and other herbivorous animals. These animals tole ate fats badly. Rabbits die soon after the addition of even sma amounts of fat to the diet unless green vegetables are given (Mellanb and Killick, '26).

In studies by Madsen, McCay and Maynard ('32-33) 6 kids die after 133 to 315 days when given a diet consisting of cellulose, starch casein, sucrose, yeast, lard, a salt mixture, and tomato juice, to whic was added 12 cc. of cod liver oil per week. The kids died unexpectedl and without obvious symptoms but autopsy showed failing circulatio intermuscular edema of the legs, increased fluid in the body cavitie congestion and edema of the lungs, and severe hemorrhagic enteriti There was fatty degeneration of the liver and kidneys. Histologica examination revealed hyaline, waxy, or Zenker's degenerative change in the muscles, with fibroblastic tissue tending to replace the deger erating muscle. The heart muscle was involved in all cases. Histo logically, local well-defined grayish areas and hyaline and granula degenerative changes were seen, with necrotic fibers in process o absorption in some areas. There was increase in the amount o connective tissue, which tended to replace portions of the degenerate muscle. Rabbits and guinea pigs, when fed the "synthetic" diet, wer injured in a similar manner by cod liver oil. No deleterious effect

were observed, however, when the cod liver oil was added to a stock diet. Madsen and associates tentatively suggested that the injurious factor is contained in the fatty acid fraction, and Golding ('28) found that the non-saponifiable fraction of cod liver oil was not injurious. In this connection it is of interest to note that Daniel and McCollum ('31) found that rats grew well and appeared healthy when fed a diet of ether-extracted menhaden meal, 14; cod liver oil, 2; butter fat, 3; agar-agar, 1; salt mixture, 51.2; dextrin, 77.9; plus the 70 per cent alcoholic extract of 10 grams of wheat germ. On the same diet modified by including 5 per cent of oleic acid, which replaced an equivalent amount of dextrin, rats were stunted in growth and were very inferior in appearance, thus indicating that fats other than cod liver oil may be injurious.

Norris and Church ('30) have shown that small doses of isoamyl amine, which is found in cod liver oil, and also choline, when given continuously, may produce paralysis, convulsions, and impairment of growth, and that these symptoms may be prevented by increasing the amount of yeast in the diet. It is suggested that these and perhaps other nitrogenous bases contained in cod liver oil may be the cause of its toxicity.

Madsen ('36) has recently reported further studies on the muscular dystrophy in rabbits and guinea pigs caused by feeding cod liver oil or a concentrate made from it. Lard or cottonseed oil fed with cod liver oil did not protect against dystrophy. With his "synthetic" diet, the replacement of cod liver oil by a concentrate fed in cottonseed oil solution, the results suggest that the cottonseed oil was protective. The basal diet without added fat, but with addition of concentrate containing the nonsaponifiable portion of cod liver oil, produced dystrophy to nearly the same extent as the same diet containing 6 per cent of lard. Substitution of cottonseed oil for the lard resulted in a high degree of protection against the muscle lesions. No such protection was afforded by this oil, however, when cod liver oil was fed with it, in place of the concentrate.

When the "synthetic" diet was supplemented with concentrate to provide vitamins A and D, and was fed with 6 per cent of either lard or cottonseed oil, the muscle lesions were seen in the lard group but the groups receiving either 3 or 6 per cent of cottonseed oil were in great measure protected. One in 10 receiving 3 per cent of this oil showed symptoms. Microscopically there were some scattered necrotic fibers of recent development but the muscles looked normal in color and consistency.

Goettsch and Pappenheimer ('31) observed degeneration of the skeletal muscles of rabbits fed a grain mixture consisting of oats 65,

barley 85, wheat 45, and wheat bran 5, supplemented with cod oil. Madsen employed this ration for guinea pigs, with and wit cod liver oil. The grain mixture alone was inadequate and para was seen in some of the animals, but the animals that received gm. of cod liver oil daily all showed paralysis and severe mu dystrophy.

From these studies it appears that the fatty acids of cod liver are toxic, and that the muscles are especially sensitive to injury fr them. It is not improbable that the organic bases in this oil are pa responsible for its effects. But the grain mixture described, with cod liver oil, caused the condition in lesser degree. The feeding cod liver oil with such natural foods as constitute an optimal rat apparently protects animals against this type of food injury. Possi the toxicity of cod liver oil is caused by its autoxidation and con quent destruction of vitamin E (Chapter XXIII).

Fats in Infant Nutrition.—Holt and his associates ('35) ha made extensive investigations on the fat metabolism of infants. Th conclude that in the normal subject fats are completely hydrolyzed the intestines. There does not appear to be a constant relation betwe total solids and fat in the feces. In infants on a low-fat diet the fec fat is largely derived from the secretions. Calcium and magnesium the food affect adversely the absorption of fat; other non-fatty food stuffs have no apparent effect. Their observation that neither the fa globule size nor the melting point of dietary fats have any influenc upon digestion and absorption in the infant is of special interest sinc emphasis on the importance of both these factors has been frequen by pediatricians. In a mixed fat, absorption is favored by fatty acid with one or more unsaturated linkages, and relatively short carbo chains. It is impaired by the long chain saturated fatty acids. The found that ketosis occurred just as quickly with odd-carbon as wit even-carbon fatty acids.

Free fatty acids, they found, irritate the intestines and soaps increas the water requirement; hence hydrolyzed fats should not be used i diarrheal cases. Ethyl esters of fatty acids are inferior to triglyceride They are saponified with difficulty, have a low percentage retentio and lead to ketosis at unusually low levels of fat absorption. Th absorption was found to be better with breast milk than with butte but these were surpassed by olein, olive oil, and soy bean oil. De termination of iodine values indicated that there is selective absorptio of individual fatty acids in mixed fats. On fat-poor diets infants ten to have loose, fermentative stools. In one case eczema and spasmodi bronchitis regularly appeared and were relieved by increasing the fa intake. Fat absorption in infancy increases slightly with age. Low fat retention in atrophic infants is not adequately explained by low

intake. It would appear that these infants have difficulty in absorbing fat.

Fat digestion (saponification) is less complete in the premature than in the full term infant. Retention of unsaponifiable material is also impaired. In premature infants olive oil and soy bean oil show much higher percentage absorption than butter fat, and substitution of these fats for milk fat is frequently followed by improvement of the weight curve. Linolein is better absorbed than olein, indicating that the presence of more than one double bond in a fatty acid chain favors fat absorption.

Sullivan and Fershtand ('35) utilized the observations on fat absorption as an index of liver function. One hundred grams of cottonseed oil were given orally to normal men, to diabetics, and to patients with various types of impaired liver function. After a 15 hour fast, blood was taken, the oil administered, and blood again drawn 3, 6, and 9 hours later. In the normal subject the total lipids rose to 65 per cent above the fasting level at 6 hours and returned to normal at 9 hours. In diabetics the same level was reached at 6 hours but there was a lag in the fall, while patients with liver disease showed but a small rise and there were no signs of a decrease even at 9 hours. Defective absorption is held responsible for this behavior.

The Effect of the Nature of Food Fat on the Composition of Body Fat.—Many studies have been reported on the effects of various feeding-stuffs on the melting point and iodine number of the fat of hogs. This work was stimulated because of the frequent occurrence of so-called "soft pork," a term applied to the hog carcasses which remain soft and flabby after being exposed in the chilling room. Such pork is unsalable. The literature has been reviewed by Hankins and Ellis ('26). Ellis and Isbell ('26) analyzed the fats of hogs fed various combinations of feeds. They found that the percentages of saturated fatty acids, oleic acid, and linoleic acid of lard from peanut- and soy bean-fed hogs were similar to the content of these acids in peanut and soy bean oils. The linoleic acid showed the most striking changes, increasing from 1.9 per cent in the lard from hogs fed brewer's rice, to 30.6 per cent in oily fat from hogs fed soy beans. There is a close relation between the food fat and the fat laid down by the hog. Anderson and Mendel ('28) have shown that the rat behaves in respect to food and body fat as does the hog. When the body fats were synthesized from starches or dextrin they were hard (i.e., they had a low iodine number and high melting point).

Absorption of Fatty Acids from the Intestine.—Experimental evidence seems to prove that the fatty acids liberated from fats in the intestine, through the agency of enzymes, are absorbed into the epithelial cells of the small intestine. Jeker ('36) has recently reinvesti-

gated this subject. By employing stains which differentiate bet neutral fats and fatty acids, he has shown that fatty acids ca demonstrated in the tips of the epithelial cells of the villi with to 20 minutes after feeding fat to rats. By the 6th hour the cell completely filled with fat droplets which are composed of neutral free fatty acids being absent. This indicates a resynthesis of neutra in the cells. Artom and Peretti ('35) administered iodized fa rabbits and found that after a short time the iodized fatty acid the intestinal mucosa are present in the neutral fats, but that 3 days a considerable portion of these are to be found in the acet insoluble fraction, i.e., in the phosphoaminolipids, the lecithins, kephalins. They have also shown that during absorption a portio the saturated fatty acids is desaturated (e.g., stearic acid conve to oleic acid). Verzár and McDougall ('36) have thoroughly discu fat absorption.

The Origin of Milk Fats.—The composition of the milk fa animals is different from the stored fats in the same animal. It cont representatives of practically all the saturated fatty acids from but acid, which is C_4-, to cerotic acid, which is C_{26}- and possibly hig The content of short-chain fatty acids is higher than in any other in the body. There is in milk a great complexity of unsaturated fa acids, which, like the saturated ones, are present in the form triglycerides. In the case of Carnivora and Omnivora, food fats n appear promptly in the milk. But in Herbivora, food fat has little eff on the composition of milk fat, which appears to be formed larg from carbohydrate. Meigs, Blatherwick and Cary ('19) made elabor studies of the source of milk fats. Their findings are: 1. That norm blood plasma contains no phosphorized proteins, and probably phosphorus compounds at all except phospholipids and inorga phosphates. 2. The precursor in plasma of milk fat and milk ph phorus is either lecithin or some related body.

Since about 20 per cent of the total fatty acids of milk fats con of fatty acids with 14 or less carbon atoms in their chains and th are notably rare in blood fats and food fats other than milk, the mo of formation of these fats is of great interest to biochemists a physiologists. A recent series of investigations by Graham, Jones a Kay ('36) has thrown new light on this question. They state t volatile fatty acids cannot be detected in blood, which is the source all the constituents of milk. Parry and Smith ('36) made an elabor study of the fats of cattle and reported the following values for fa acids:

	Ox Depot Fat %	Ox Blood Fat %	Ox Liver Fat		Pig Liver Fat %
			Phosphatide %	Glyceride %	
Saturated acids					
Lower acids . . .	6	*	Trace	Trace	1
C_{16}	27	10	12.5	25	14
C_{18}	22	13	27	20	19
Higher acids . . .	Trace	3	Trace	Trace	2
Total	55	26	39.5	45	36
Unsaturated acids					
C_{16}	..	*	5	9	1.5
C_{18}	45	26	27	37	33
C_{20}	..	33	18	8	20
C_{22}	..	10	10.5	1	7.5
Total	45	69	60.5	55	62

* There was not sufficient material available to make possible the detection of small amounts of lower acids.

Hilditch and Paul ('36) give the following data relative to the component fatty acids of cow milk fat:

Component Fatty Acids of Cow Milk

Acid	Weight Percentages			Molar Percentages		
	(a)	I	II	(a)	I	II
Butyric	3.7	3.7	3.7	9.8	9.8	9.7
n-Hexanoic	2.0	2.0	2.0	4.1	4.1	4.2
n-Octanoic	1.3	1.0	0.9	2.1	1.6	1.5
n-Decanoic	2.7	2.6	2.6	3.7	3.5	3.5
Lauric	4.0	1.7	2.7	4.8	2.0	3.2
Myristic	7.9	9.3	8.6	8.2	9.6	8.9
Palmitic	23.8	25.4	26.2	21.9	23.4	24.0
Stearic	10.7	10.7	10.7	8.9	8.9	8.8
,, arachidic	0.5	0.4	0.5	0.3	0.3	0.3
,, decenoic	...	0.2	0.5	...	0.3	0.6
,, tetradecenoic . . .	...	1.2	0.7	...	1.3	0.8
,, hexadecenoic . . .	...	5.0	4.9	...	4.6	4.5
,, oleic	38.3	32.4	31.9	31.9	27.0	26.5
,, octadecadienoic . .	4.7	4.0	3.9	4.0	3.3	3.3
,, C_{20-22} unsaturated . .	0.4	0.4	0.2	0.3	0.3	0.2

(a) Without allowance for unsaturated acids below C_{18}.

From a study of oxidation products of the unsaturated fatty
of milk Hilditch and Paul conclude that in the principal unsatu
fatty acids in the glycerides of milk fat the position of the eth
bond in the three acids below oleic is the same as in that acid:

$CH_2 : CH \cdot (CH_2)_7 \cdot COOH$	Decanoic acid
$CH_3 \cdot (CH_2)_3 \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$	Tetradecenoic acid
$CH_3 \cdot (CH_2)_5 \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$	Hexadecenoic acid
$CH_3 \cdot (CH_2)_7 \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$	Oleic acid

These investigators suggest that the lower-saturated glyceride
milk, which contain the volatile fatty acids, are formed in the m
mary gland by shortening the carbon chains of preformed
glycerides, and the minor lower-unsaturated components just m
tioned may represent degradation products of oleo-glycerides w
have escaped complete saturation to lower-saturated groups.
hypothesis, which is supported by much experimental data, supp
the shortening of the carbon chain by oxidative removal of
terminal CH_3 group with subsequent reduction of the new term
carbon atom. It is implied that the oxidation and reduction of
fatty acids occur while in combination with glycerol, thus offering
explanation for the high content of fully saturated glycerides
milk fat.

The Behavior of Certain Fatty Acids in the Mammary Gland. Hilditch and Thompson ('36) have reported some instructive stud
on the effects of feeding certain fatty acids (in the form of glyceride
to lactating cows, with the objective of identifying the components
cod liver oil which cause lowering of the fat content of milk.

Linseed oil has a higher mean unsaturation than most cod li
oils, this being due to glycerides which contain linoleic and linole
acids,

$CH_3 \cdot (CH_2)_5 \cdot CH : CH \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$	Linoleic aci
$CH_3 \cdot (CH_2)_3 \cdot CH : CH \cdot CH : CH \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$	Linolenic a

each of which forms about 40 per cent of the total fatty acids of linse
oil. These are 18-carbon acids. Rape seed oil contains nearly half
weight of erucic acid glycerides. Erucic acid contains 22 carbon ato
in its chain:

$CH_3 \cdot (CH_2)_7 \cdot CH : CH \cdot (CH_2)_{11} \cdot COOH$ Erucic acid

Cod liver oil contains a high percentage of tetra- and penta-ethn
unsaturated fatty acids of 20- and 22-carbon acids.

The effect of these fatty acids on the composition of the fats of m
is shown in the following table:

COMPONENT ACIDS OF COW MILK FATS AS AFFECTED BY FOOD FATS

ACID	MOLAR PERCENTAGES			
	Control	Linseed	Rape	Cod Liver
utyric	11.5	11.2	9.9	5.7
-Hexanoic	4.3	4.1	3.4	1.2
-Octanoic	3.7	2.1	2.3	1.6
-Decanoic	5.0	3.1	2.2	1.9
auric	5.0	3.6	3.4	5.1
Iyristic	10.8	8.6	9.1	7.4
almitic	20.5	20.0	17.0	22.8
tearic	10.1	8.2	11.7	7.3
s arachidic	0.5	0.4	0.4	0.5
leic	23.3	32.8	34.0	38.3
s octadecadienoic	4.5	5.0	3.2	4.1
s C_{20-22} unsaturated	0.8	0.9	0.8	4.1
s erucic	...	...	2.6	...

From the above table it is apparent that feeding cod liver oil educed the lower-saturated fatty acids of the milk fat by one-half; he content of oleic acid is greatly increased, and the content of nsaturated acids of C_{20-22} is five times the normal. It is suggested hat selective adsorption of the highly unsaturated fatty acids by the nzymes responsible for the elaboration of typical cow milk fats etards the normal enzymatic function. These newer researches reatly extend our knowledge of the types of chemical reactions which ke place in the mammary gland, while milk secretion is in progress. ince a relatively small amount of the total milk obtained is in the rgid gland before milking starts and the gland forms most of the sein, fats, and milk sugar of milk during the short interval required remove it in the milking process, the rapidity with which the rmation of milk takes place in a high producing cow (yielding as uch as 40,000 lbs. of milk a year) is truly phenomenal. A cow elding 100 lbs. of milk daily, in three milkings, would transform ore than half of the pound or more of fats yielded in one milking to modified fatty acids during an interval of a few minutes.

The Effects of Feeding Cholesterol to Animals.—When cholesrol, dissolved in oil, is fed to rabbits or guinea pigs there is a deposion of cholesterol esters (cholesterol-fatty acids) in the aorta, producg a condition simulating atherosclerosis as seen in human subjects. his has not been observed in most laboratory animals. Duff ('35) afords good experimental evidence that there is marked change in the rmeability of the arterial walls and that anatomical damage and depsition of lipids, especially cholesterol esters, results from this rather

than from hyperlipemia and hypercholesterolemia. Thyroidec causes similar changes in the arterial walls. There is no correl however, between the blood cholesterol values and the degr atherosclerosis in human accident cases, which strongly indicates the level of this substance is not the primary cause of the disease h may be a contributing factor. Elliot and Nuzum ('36) and Page associates ('36) have shown that there is no apparent relationshi tween the concentration of the various blood lipids and the occurr of essential hypertension in man. Sperry and Schick ('36) report certain cases of xanthomatosis (a new growth in the skin consistin connective tissue undergoing partial fatty degeneration) are impr by excluding from the diet cholesterol-rich foods. Other cases not benefited. It is of interest that dogs deprived of magnesium a high cholesterol ester content in their blood (Kruse, Orent McCollum, '33).

Best and Ridout ('36) have recently reported the effects of cho feeding on the cholesterol ester content of the liver in rats in wh fatty liver has been produced by feeding cholesterol. They found the addition of choline causes a very definite fall in both glyceride cholesterol ester content of the liver. Such fatty liver is produced feeding a diet containing 2 per cent of cholesterol. When this h intake of cholesterol exists, choline administration may cause a in the glyceride level while the cholesterol esters are increasing, later the effects of choline come into prominence.

REFERENCES

Burr and Burr (1929). J. Biol. Chem., *82,* 345; ibid. (1930), *86,* 587.
Burr, Burr and Miller (1932). J. Biol. Chem., *97,* 1.
Turpeinen (1938). J. Nutr., *15,* 351.
Spadola and Ellis (1936). J. Biol. Chem., *113,* 205.
Brown and Burr (1936). J. Biol. Chem., *114,* xvi.
Sinclair (1936). J. Biol. Chem., *114,* xciv.
Sinclair (1932). J. Biol. Chem., *96,* 103.
Wesson and Burr (1931). J. Biol. Chem., *91,* 525.
Wesson (1927). J. Biol. Chem. *73,* 507.
Miyazaki and Abelin (1924). Biochem. Ztschr., *149,* 109.
Aberlin (1926). Klin. Wchnschr., *5,* 367.
Wesson and Murrell (1934). Proc. Soc. Exper. Biol. & Med., *31,* 11
Hansen (1933). Proc. Soc. Exper. Biol. & Med., *30,* 1198.
Epstein and Glick (1937). Archiv. Dermat. & Syphilology, *35,* 427.
Brown, Hansen, Burr and McQuarrie (1938). J. Nutr., *15,* Suppl.,
Schantz, Elvehjem and Hart (1938). J. Biol. Chem., *122,* 381.
Deuel, Gulick and Butts (1932). J. Biol. Chem., *98,* 333.
Clark and Murlin (1936). J. Nutr., *12,* 469.
Deuel, Hallman, Butts and Murray (1936). J. Biol. Chem., *116,* 6

Whipple and Church (1935). J. Biol. Chem., *109*, xcviii.
Evans and Lepkovsky (1929). J. Biol. Chem., *83*, 269.
Evans, Lepkovsky and Murphy (1934). J. Biol. Chem., *107*, 439.
Hershey and Soskin (1931). Am. J. Physiol., *98*, 74.
Best and Huntsman (1932). J. Physiol., *75*, 405
Best, Hershey and Huntsman (1932). J. Physiol., *75*, 56.
Best and Channon (1935). Biochem. J., *29*, 2651.
Fletcher, Best and Solandt (1935). Biochem. J., *29*, 2278.
Bloor, Okey and Corner (1930). J. Biol. Chem., *86*, 291, 307.
Klem (1935). Hvalradet Skrifter, No. 2, 49; cited in Ann. Rev. Biochem., *5*, 227 (1936).
Terroine and Trimbach (1934). Arch. internat. de Physiol., *39*, 377.
Bloor and Snider (1934). J. Biol. Chem., *107*, 459.
Sinclair (1934). Physiol. Rev., *14*, 351.
Boyd (1935). J. Biol. Chem., *111*, 667.
Agduhr (1926). Acta Paediat., *5*, 319; *6*, 165; *7*, 289.
Harris and Moore (1929). Biochem. J., *23*, 1114.
Harris and Innes (1931). Biochem. J., *25*, 367.
Mellanby and Killick (1926). Biochem. J., *20*, 902.
Madsen, McCay and Maynard (1932-33). Proc. Soc. Exper. Biol. & Med., *30*, 1434.
Golding (1928). Proc. World's Dairy Congress.
Daniel and McCollum (1931). U. S. Dept. of Commerce, Bureau of Fisheries, Investigation Report No. 2.
Morris and Church (1930). J. Biol. Chem., *89*, 437.
Madsen (1936). J. Nutr., *11*, 471.
Goettsch and Pappenheimer (1931). J. Exp. Med., *54*, 145.
Holt, Tidwell, Kirk, Cross and Neale (1935). J. Pediat., *6*, 427; Tidwell, Holt, Farrow and Neale (1935). J. Pediat., *6*, 481.
Sullivan and Fershtand (1935). Arch. Internal Med., *55*, 834.
Hankins and Ellis (1926). U. S. Dept. Agric., Bull. 1407.
Ellis and Isbell (1926). J. Biol. Chem., *69*, 219.
Anderson and Mendel (1928). J. Biol. Chem., *76*, 729.
Ecker (1936). Arch. f. d. ges. Physiol., *237*, 1.
Artom and Peretti (1935). Arch. internat. de Physiol., *42*, 61.
Verzár and McDougall (1936). "Absorption from the Intestine." Longmans, Green & Co., London.
Meigs, Blatherwick and Cary (1919). J. Biol. Chem., *37*, 1.
Graham, Jones and Kay (1936). Proc. Roy. Soc. (London), B, *120*, 330.
Harry and Smith (1936). Biochem. J., *30*, 592.
Hilditch and Paul (1936). Biochem. J., *30*, 1905.
Hilditch and Thompson (1936). Biochem. J., *30*, 677.
Duff (1935). Arch. Path., *20*, 81.
Elliot and Nuzum (1936), Arch. Internal Med., 57, 63.
Page, Kirk and Van Slyke (1936). J. Clin. Invest., *15*, 109.
Perry and Schick (1936). Am. J. Dis. Child., *51*, 1372.
Kruse, Orent and McCollum (1933). J. Biol. Chem., *100*, 603.
Best and Ridout (1936). J. Physiol., *86*, 343.

CHAPTER

V

General Nature of Proteins and Amino Acids and Their Significance in Nutrition

IT SHALL BE the purpose in this chapter and the one to foll to review and summarize the most pertinent evidence concern the nutritional significance of proteins and amino acids, but it w not be possible to furnish a comprehensive treatment owing to t lack of space.

Investigations in the field of protein nutrition have had as th objectives the solution of many problems, some of which are: (a) t number and nature of the amino acids contained in proteins; (b) t amounts of each of these yielded by different proteins; (c) the vario functions of proteins and their amino acids in the animal body; (d the mechanisms of protein metabolism; (e) the mechanisms of prote formation in both the animal body and plant; (f) the factors affecti protein digestion and utilization; (g) the ability of tissues to sy thesize amino acids; (h) the precursors of amino acids synthesize in the body; (i) the protein and amino acid requirements for vario life functions, e. g., growth, maintenance, reproduction, and lactatio and (j) the most economical and physiologically satisfactory dieta combination of proteins and amino acids to promote the desired nut tional status.

General Functions of Proteins in the Body.—It is difficult to sta a definite time in history when anyone first became concerned abo the nature of proteins and their biological functions. Mulder (1839 a Dutch chemist, definitely recognized the general similarity betwee plant and animal proteins and indicated some appreciation of th rôles of proteins in bodily functions. He had the distinction of coinin the name protein (from the Greek word, proteios, meaning primary Gradually the concept developed that proteins are indispensab dietary factors which are necessary for the formation of tissue protei

nd the replacement of nitrogenous compounds lost in metabolism. his is clearly evident in the theories of Liebig, Pflüger, and Voit con-erning the mechanism of protein metabolism, which will be consid-ered subsequently.

As early as 1866 it was learned that proteins are not essential as a ource of energy if carbohydrate and fat are furnished in sufficient mount (Pettenkofer and Voit), but a realization that the functions f protein cannot be satisfied without regard to the amino acid com-osition of dietary protein was not appreciated before 1872. At that ime Carl Voit found that gelatin could not be substituted for meat rotein in the diet of dogs without the loss of body protein. By the eginning of the twentieth century the view had become generally ccepted that the dietary functions of protein could not be served ithout regard to the "nutritive quality" of the constituent amino cids.

During the past thirty or forty years, and particularly the last ifteen years, we have witnessed marked progress in the chemistry nd physiology of proteins. At present it appears that all enzymes nd several of the hormones are protein in composition. Even viruses nd bacteriophage, as yet mysterious indeed in function, have been hown, in some instances, to have the general nature of protein. Thus, n addition to the well-recognized role of proteins in the structure f various tissues, particularly muscle, the maintenance of fluid bal-nce, and the mechanism of respiration and neutrality regulation, pro-eins are components of specific bodily activities which include those f enzymes, hormones, and viruses.

At present the following enzymes are known to be simple pro-eins: urease, pepsin, trypsin, chymotrypsin, and carboxypeptidase. In ddition, Kunitz and Northrop ('33) have prepared crystalline hymotrysinogen and pepsinogen, and have shown that they are rotein in nature. Also, Theorell ('34) has crystallized the yellow xidation enzyme of Warburg and Christian, and found it to be protein with adsorbed riboflavin. Certain enzymes, as for example atalase, are composed of a protein combined with a prosthetic roup.

The definition of an enzyme as a catalyst, which is elaborated by ving cells and which may act independently of the life processes of he cells, still holds in the light of recent researches, but the older heory that the proteins acted as carriers of the actual enzymes, must, seems, be discarded. One cannot say at present, however, that all nzymes are protein, since emulsin is not inactivated by pepsin or rypsin.

Theories of Protein Metabolism.—It is appropriate to give some ttention to protein metabolism, since one can scarcely contemplate

any nutritional aspect of proteins and amino acids without refe to their metabolism. The subject is deeply grounded in histor it is helpful to review certain aspects of it here.

Definite ideas of protein metabolism have existed ever sinc time of Liebig. Voit's theory, which replaced the outmoded vie Liebig, was derived from metabolic experiments with dogs. H served that a well-fed dog, subjected to fasting, excreted, at fir abundance of nitrogenous waste products, but as the fast conti very little nitrogenous matter was excreted in the urine. This sugg to Voit that a marked destruction of circulating protein occurs time, during fasting, until this reserve is reduced to a low level. nitrogen excreted after reaching this low level was believed to its origin in the organized tissues. Voit attributed to the cells function of utilizing proteins, believing that the circulating protei drawn into the cells for transformation into degradation prod which later appear in the urine.

Voit's theory, first formulated in 1867, was criticized by Pflüge 1893, who presented a new one in its place. Pflüger held that pro must become living protoplasm before it can be utilized for the ne of the body. His pupil, Schöndorff, perfused the blood of a starv dog through its hind limbs, and the liver of a well-fed dog, and fou an increase in the excretion of urea. But when the blood of a w fed dog was perfused through the same channels as those of starving dog the output of urea was not increased. This indica that the determining factor in protein destruction is the state nutrition in the tissue cells and not the circulating protein. Physio gists were not satisfied with Pflüger's hypothesis, since it was cl from Voit's experiments that very large quantities of protein may metabolized with great rapidity, as shown by the large output urinary nitrogen following the ingestion of a high-protein meal. It v difficult to believe that living protoplasm could be synthesized rapidly, and be almost immediately destroyed.

Folin ('05) possessed the genius to solve the problem in its ma outlines, and his interpretation of the mechanism of protein met olism is almost universally accepted. He devised analytical procedu which were less time-consuming and more accurate than any hithe available for the estimation in small amounts of all the principal co stituents of urine, together with methods for the estimation of th types of sulfur compounds which it contains. By means of th methods complete analyses were made of urines from subje (human) given a high protein diet, followed by a period of low p tein consumption. The differences, as influenced by the protein inta between the daily urines of two individuals (A and B) are illustrat by the following record:

	High-protein Diet		Low-protein Diet	
	A	B	A	B
otal nitrogen	14.8 gm.	18.2 gm.	4.8 gm.	8.0 gm.
rea nitrogen	86.3%	89.4%	62.0%	80.4%
mmonia nitrogen	3.3%	5.1%	4.2%	11.4%
reatinine nitrogen	3.2%	4.5%	5.5%	11.1%
ric acid nitrogen	0.5%	1.0%	1.2%	2.4%
ndetermined nitrogen	2.7%	5.3%	4.8%	14.6%
otal sulfur	3.11 gm.	3.73 gm.	1.04 gm.	1.65 gm.
norganic sulfur	89.4%	87.2%	60.6%	83.6%
thereal sulfur	6.5%	7.2%	11.5%	8.4%
Neutral" sulfur	4.1%	5.5%	27.9%	8.0%

On the basis of his studies, Folin deduced the following generalizations:

1. The absolute amount of creatinine eliminated by a man on a eat-free diet (meat contains creatinine) is a constant quantity, different for different individuals, but independent of the amount of otein metabolized, and hence, of the nitrogen eliminated.

2. When the total amount of protein metabolized is greatly reced, the absolute quantity of uric acid excreted is diminished, but t nearly in proportion to the diminution in total nitrogen eliminated, d the per cent of uric acid nitrogen in the urine in terms of the tal is much increased.

3. When the amount of protein metabolized is small, and consequently the output of nitrogen in the urine is greatly reduced, there usually, but not necessarily, a decrease in the output of ammonia. pronounced reduction of the total nitrogen is, however, always companied by a relative increase in the ammonia-nitrogen, proded the food does not yield an alkaline ash.

4. With pronounced diminution of the total nitrogen eliminated, ere is a corresponding reduction in the per cent of the total nitrogen the urine represented by urea. When the total daily nitrogen excren is reduced to 3 to 4 gm., about 60 per cent of it only is in the rm of urea.

5. The inorganic sulfate excretion, in percentage of the total, falls hen there is diminution of the total sulfur output.

6. The neutral sulfur elimination is analogous to that of creatinine. represents products which are, in the main, independent of the tal amount of sulfur eliminated, and hence, of protein metabolized.

7. Ethereal sulfates in the urine represent a form of sulfur metab-

olism which becomes more prominent when the diet provides or no protein.

On the basis of his data Folin reasoned that protein metab must be of two different kinds. One is extremely variable in qua whereas the other remains nearly constant. The first yields c urea and inorganic sulfates, no creatinine, and probably no ne sulfur. The second, or constant catabolism, is largely represente creatinine and neutral sulfur, and to some degree by uric acid ethereal sulfates. As the total catabolism of protein is reduced, the prominent becomes the type represented by urea and inorganic fates. While urea and inorganic sulfates represent chiefly the var catabolism, the possibility is not precluded that they also represe some extent the constant type of metabolism.

Folin distinguished the constant type of protein catabolism as "endogenous," in contrast with the "exogenous" type. The for represents the protein transformations involved in metabolism, ch oxidative, of living tissues; the latter represents the hydrolytic cleav and rapid degradation of protein digestion products into urea, organic sulfates, etc.

The physiological state represented by the constant type of pro in metabolism will remain nearly unchanged only when suffici carbohydrate is ingested to maintain energy equilibrium. In the sence of sufficient carbohydrate for this purpose, protein degradati at the expense of tissue protein, will take place, about 58 per cent the carbon being converted into carbohydrate.

Therefore in the absence of sufficient carbohydrate, as was sho by Ringer (1912), an additional amount of protein is essential, abo the "wear and tear" quota. This portion of dietary protein can spared by carbohydrate, but not by fats, since all efforts to dem strate the formation of glucose from fats have thus far failed.

Folin and Denis (1912) applied their analytical methods for determination of urinary constituents to the elucidation of the me anism of protein metabolism. They clearly demonstrated that, amino acids are absorbed from the gut, their concentration first ri in the portal blood, then in the liver, and as the blood passes fr the liver into the systemic circulation, the latter shows a high tide amino acids. The high level in the blood soon falls, while that of muscles and organs rises, showing that these tissues remove ami acids from the blood. Simultaneously with the flooding of the liv systemic blood, and tissues with amino acids, the formation of u begins, and continues until the blood and tissue content of these ac returns to the fasting level, after absorption from the gut is complet Van Slyke and Cullen confirmed Folin and Denis' findings in ev

detail, using Van Slyke's direct method for estimating amino acids.

The significance of the "deposit protein" which is retained for a time after high-protein feeding, and which puzzled the older investigators, is now clearer. It is not protein, but amino acids, held in the tissues, which form a reservoir. The lag extending over several days, when extreme changes are made in the protein intake, is explained by the existence of this reservoir.

The origin and significance of "neutral sulfur" and creatinine are still obscure. It seems necessary to conclude that they arise through certain reactions in the body which proceed at constant velocity, and are not associated with the rate of metabolism of ingested foodstuffs. Urea and sulfate sulfur, on the other hand, are proportional to the amount of catabolism, both of ingested protein which never becomes involved as part of the structure of living tissues, and of tissue proteins as well.

General Properties of Amino Acids.—When proteins are subjected to appropriate hydrolytic action, amino acids may be isolated in pure form from the hydrolysate. It is uncertain how many amino acids exist in nature, combined as protein, but the number is certainly greater than 20.

The following table, modified in the light of recent researches, is taken from the extensive monograph of Vickery and Schmidt ('31). It shows the names of the known amino acids, derived from protein, and the dates and names of their discoverers.

Amino Acid	*Earliest Observation of the Amino Acid as a Product of Hydrolysis of Proteins*	
Glycine	Braconnot	1820
Leucine	Braconnot	1820
Tyrosine	Bopp	1849
Serine	Cramer	1865
Glutamic acid	Ritthausen	1866
Aspartic acid	Ritthausen	1868
Phenylalanine	Schulze and Barbieri	1881
Alanine	Weyl	1888
Lysine	Drechsel	1889
Arginine	Hedin	1895
Iodogorgoic acid	Drechsel	1896
Histidine	Kossel, Hedin	1896
Cystine	Mörner	1899
Valine	Fischer	1901
Proline	Fischer	1901
Tryptophane	Hopkins and Cole	1901
Oxyproline	Fischer	1902
Isoleucine	Ehrlich	1903
Thyroxine	Kendall	1915
Hydroxyglutamic acid	Dakin	1918
Methionine	Mueller	1922
Threonine	Rose	1935

Several other amino acids have been reported to be derived proteins but they have been studied by a single investigator These include the following:

Dibromotyrosine	Mörner	1913
Norleucine	Thudichum, Abderhalden and Weil	1901 1913
Hydroxyaminobutyric acid	Schryver and Buston	1926
Hydroxyvaline	Schryver and Buston	1926
Hydroxylysine	Schryver and Buston	1925
Protoctine	Schryver and Buston	1926
Dihydroxyphenylalanine	Guggenheim	1913
Citrulline	Wada	1930
Djenkolic acid	Van Veen and Hyman	1935
Canavanine	Kitagawa	1929

The structure of the amino acids is shown by the following mulae:

I. Glycine, or amino-acetic acid: $CH_2(NH_2)\cdot COOH$

Alanine, or α-amino-propionic acid:

$$CH_3\cdot CH(NH_2)\cdot COOH$$

Valine, or α-amino-isovalerianic acid:

$$\begin{matrix} CH_3 \\ \diagdown \\ \quad CH\cdot CH(NH_2)\cdot COOH \\ \diagup \\ CH_3 \end{matrix}$$

Leucine, or α-amino-isocaproic acid:

$$\begin{matrix} CH_3 \\ \diagdown \\ \quad CH\cdot CH_2\cdot CH(NH_2)\cdot COOH \\ \diagup \\ CH_3 \end{matrix}$$

Isoleucine, or α-amino-β-methyl-β-ethyl-propionic acid:

$$\begin{matrix} CH_3 \\ \diagdown \\ \quad CH\cdot CH(NH_2)\cdot COOH \\ \diagup \\ C_2H_5 \end{matrix}$$

Norleucine, or α-amino-caproic acid:

$$CH_3\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$$

II. Amino dicarboxylic acids:

Aspartic acid, or α-amino-succinic acid:

$$HOOC\cdot CH_2\cdot CH(NH_2)\cdot COOH$$

Glutamic acid, or α-amino-glutaric acid:

$$HOOC\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$$

III. Hydroxy-amino acids:

Serine, or α-amino-β-hydroxy-propionic acid:

$$CH_2OH \cdot CH(NH_2) \cdot COOH \quad (l-)$$

Threonine, or α-amino-β-hydroxy-butyric acid:

$$CH_3 \cdot CHOH \cdot CH(NH_2) \cdot COOH \quad [d(-)]$$

Hydroxyglutamic acid, or α-amino-β-hydroxy-glutaric acid: $HOOC \cdot CH_2 \cdot CHOH \cdot CH(NH_2) \cdot COOH$ (d-)

IV. Amino acids with aromatic nuclei:

Phenylalanine, or α-amino-β-phenyl-propionic acid:

$$C_6H_5 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \quad (l-)$$

Tyrosine, or α-amino-β-p-hydroxyphenyl-propionic acid: $HO \cdot C_6H_4 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$ (l-)

Iodogorgoic acid, or 3, 5-diiodotyrosine:

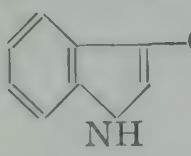
$$HO \cdot C_6H_2I_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \quad (?-)$$

Thyroxine, or β-[3, 5-diiodo-4-(3′, 5′-diiodo-4′-hydroxyphenyl)phenyl]-α-amino-propionic acid:

$$HO \cdot C_6H_2I_2\text{-}O\text{--}C_6H_2I_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \quad (l-)$$

V. Amino acid with an indole nucleus:

Tryptophane, or β-indole-α-amino-propionic acid:



(l-)

VI. Amino acids containing sulfur:

Cystine, or dicysteine, or di-(β-thio-α-amino-propionic acid):

$$HOOC \cdot CH(NH_2) \cdot CH_2 \cdot S\text{--}S \cdot CH_2 \cdot CH(NH_2) \cdot COOH \quad (l-)$$

Methionine, or α-amino-γ-methylthiol-butyric acid:

$$CH_3 \cdot S \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \quad (l-)$$

Djenkoic acid, or methylene-di-[β-thiol-α-amino-propionic acid]:

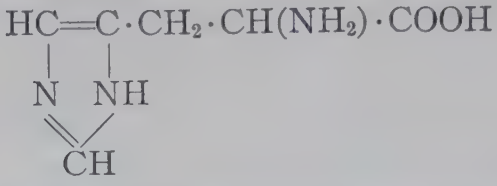
$$CH_2[S \cdot CH_2 \cdot CH(NH_2) \cdot COOH]_2$$

VII. Basic amino acids:

Lysine, or α, ϵ-diamino-caproic acid;

$$H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \quad (d-)$$

Histidine, or β-imidazole-α-amino-propionic acid:



Arginine, or δ-guanidine-α-amino-valeric acid:

$H_2N \cdot C(: NH) \cdot NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$

Ornithine, or α, ε-diamino-valeric acid, (not derived from proteins):

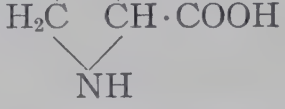
$H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$

Citrulline, or δ-carbamide-α-amino-valeric acid, (isolated from melon juice):

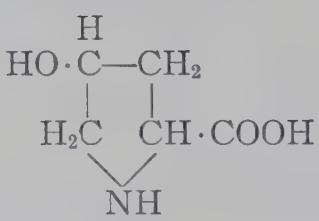
$H_2N \cdot CO \cdot HN \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$

VIII. Amino acids with pyrrole nuclei:

Proline, or α-pyrrolidine carboxylic acid:



Hydroxyproline, or γ-hydroxy-α-pyrrolidine carboxylic acid:



It is evident from the tables that the general formula, R-(NH_2)COOH, is common to all the amino acids except proline a hydroxyproline. Since all amino acids contain both acidic and b groups, they are amphoteric substances. Certain of the amino ac have rather unique structures, as for example, tyrosine, which i substituted phenol. Thus it is relatively easy to isolate some of amino acids in pure form, but most of them are difficult to iso and purify owing to the lack of sufficient differences between th in chemical properties.

Emil Fischer demonstrated that the monoamino acids, after c version into their esters, can be fractionally distilled *in vacuo*. T use of butyl alcohol as a means of isolating the monoamino acids fr

ner protein hydrolysates was discovered by Dakin. The method is considerable advantage since it permits the separation of amino ds into three groups: monoamino acids insoluble in dry butyl alco-l; proline and hydroxyproline soluble in dry butyl alcohol; and basic and dicarboxylic acids insoluble in wet butyl alcohol.

Various other procedures are employed for the isolation of these bstances. They include the use of heavy metals, as precipitants, and ctrolytic methods. The separation of the basic amino acids by ans of electrolysis was made practicable by Foster and Schmidt. ethods have been devised for the synthesis of all the amino acids t even the best procedures for certain ones are too expensive for eir production in sufficient amounts to be useful in nutritional re-arch. Moreover, all the amino acids, except glycine, are optically ive. The synthetic products are racemic mixtures and, therefore, nerally unsuitable for many types of nutritional investigation as ll as general dietary uses. This is owing to the almost complete ability of the body to utilize amino acids which are optically un-tural. The development of economical methods for the isolation natural amino acids in pure form will constitute an important ntribution. Without such methods nutritional and physiological in-stigations of amino acids will continue to be greatly hampered.

Structure of the Proteins.—Since the amino acids may be formed acid hydrolysis of proteins, it is inferred that the former are linked gether in chains through abstraction of a molecule of water from amino group of one, and a carboxyl group of another:

$$\underset{\text{Glycine}}{N\cdot CH_2\cdot COOH} + \underset{\text{Glycine}}{HHN\cdot CH_2\cdot COOH} \cdots\cdots \rightarrow \underset{\text{Glycylglycine}}{H_2N\cdot CH_2\cdot CO\cdot HN\cdot CH_2\cdot COOH}.$$

The general correctness of this view is shown by the fact that thods have been developed whereby such complex *peptides,* di-, tetra-, etc., can be prepared from amino acids. These are hydro-ed, or digested, into their constituent amino acids on treatment with propriate enzymes. Mild hydrolysis of proteins with acids leads to formation of peptides, and they have been many times isolated m digestion mixtures where the hydrolyzing agent is an enzyme. ere can be no question about the general nature of protein structure ng represented by such polypeptide patterns.

On estimating quantitatively the yields of different amino acids n different proteins, such as gelatin, blood fibrin, casein, zein, din, etc., it is found that these vary greatly. Only fifteen amino ds have been found in gelatin, other proteins yield twenty or re but in very different amounts. They consist of long chains of

amino acids in peptide union. Studies of proteins by physical m demonstrate they they exist in the form of polypeptide rows, so as to make the molecules compact. Apparently these rows ar together by secondary valences.

In this brief introduction to a discussion of proteins and acids in their relation to nutrition, we are especially concerned the yields of amino acids, since these constitute the "building s for the construction of tissue proteins. It is largely upon these that the nutritive value of proteins depends. Students who des learn the technic by which present-day investigators are making ress in determining the structure of protein, viz., the order in individual amino acids are linked in the protein molecule, are ref to the recent papers by Bergmann and his coworkers (1936). ever, in view of the recent fundamental progress in protein stru it is profitable, even in a textbook of nutrition, to include son the information available at present concerning the subject.

The classical studies of Emil Fischer on the synthesis of peptides demonstrated that protein molecules are essentially pe chains composed of many amino acid units. The differences in bility, temperatures of coagulation, and yields of the various a acids, show that proteins from diverse sources are of different position. Serological studies have shown that each species synthe characteristic and specific proteins from the heterogenous mixtur amino acids which are absorbed from the digestive tract after digestion of "foreign" food proteins.

The possible number of combinations of the 22 known a acids in peptide chains is astronomical in magnitude. It is desi that we understand the properties of protein molecules from diffe sources, as expressed in molecular weight, amino acid composi arrangement of the amino acids in the peptide chains, and config tion of the molecules. Significant advances have been made in re years in these directions.

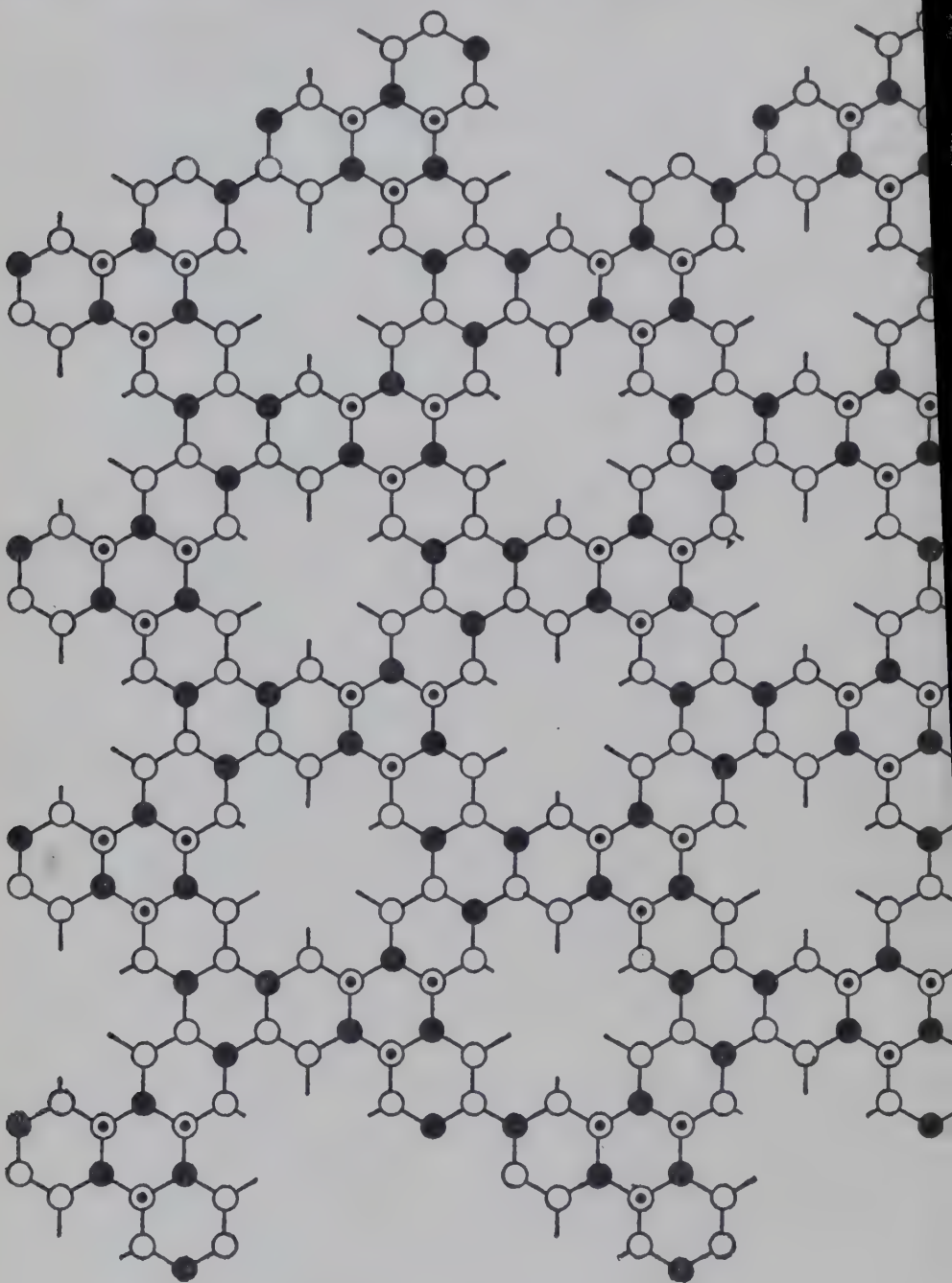
Svedberg ('34) perfected the technique of determining the mol lar weights of proteins by observing the rate at which they sedi in centrifugal fields. With the ultracentrifuge, which he perfe solutions can be subjected to forces up to 400,000 times gravity. Co dal systems, like gold sols, are by this procedure shown to con a continuous series of particles of varying mass and form. A pro solution, on the other hand, is either homogeneous with regar particle mass and shape, or contains a limited number of par species of different mass and shape. Each species proves to be ho geneous in these respects. The measurement of molecular sedime tion in centrifugal fields shows that most of the native proteins homogeneous as regards molecular weight within certain pH ra

ach protein has a characteristic pH stability region. When the bor-
rs of the stability ranges are exceeded, disintegration or aggregation
kes place. At pH 11 and beyond, disintegration of the protein
olecule occurs. At a certain degree of acidity in the solution de-
turation and aggregation to large particles occur. Many proteins
ssess, in the pH range of stability, molecular weights which are
ry great, but when brought to a certain degree of alkalinity char-
teristic of the protein, they dissociate into fragments having a molec-
ar weight of about 34,500. Thus the hemocyanin, i. e., copper
spiratory pigment, of the horseshoe crab exists in four components
molecular weights of 2,600,000; 1,300,000; 325,000 and 108,000,
thin the range from pH 5 to 10. Amandin, a globulin from the
te, which has a molecular weight of 208,000 under conditions of
bility, decomposes into fragments of about 34,500, or one-sixth the
olecular weight at pH 12. The molecular weight of ovalbumin is
500, and most of the homogeneous proteins have been shown by
e sedimentation method to possess molecular weights of 34,500, or
mple multiples of this number. Gelatin is not homogeneous but
nsists of particles varying from 10,000 to 75,000 in molecular weight.
yckoff ('36) found the molecular weight of Felton pneumococcic
tibody to be 500,000. Beard and Wyckoff ('37) found the molecular
ight of the heavy protein from virus-inducing rabbit papilloma to
in excess of 20,000,000. Virus proteins appear to have extraordi-
rily high molecular weights (as shown by Stanley and Wyckoff,
). Svedberg views the protein molecule as globular in form.

That proteins are not made up of polypeptides of every conceivable
gth is shown by their molecular weights. The fact that different pro-
ns contain, in peptide linkage, amino acids of different molecular
ights, accounts for the observed deviation from 32,000 to 35,000 for
characteristic dispersed fragments in the upper pH region of
tability.

Wrinch ('37) has proposed the cyclol theory of protein structure
account for the observed properties of proteins brought to light by
owfoot's ('35) X-ray analysis of these substances, and by the struc-
e of unimolecular films, multilaminar proteins, and globular struc-
e proposed by Svedberg. According to this theory each space-enclos-
molecule of protein consists of a piece of cyclol fabric composed of
lypeptides folded into ring structures, and held together by bonds,
nature of which, i. e., whether hydrogen bonds, "cyclol links," or
droxyl bonds, has not been decided. She conceives this network to lie
the surface of an imaginary truncated tetrahedron, and to possess
onfiguration with four triangular faces and four hexagonal faces.
is polyhedral configuration is in agreement with Svedberg's "globu-
" molecule, and accounts for its globularity. Wrinch illustrates the

cyclol fabric pattern of insulin, which consists of a molecule cont
288 amino acid residues, by the following picture.



Bergmann and coworkers have made some progress in deterr
ing the order of arrangement of certain of the amino acids in pep
chains. The following table shows the molecular weights of
proteins, and the numbers of amino acids which are present in t
molecules. All the amino acids included in this table can be
mated with considerable exactness.

HE NUMBER OF AMINO ACID RESIDUES IN THE MOLECULES OF CATTLE HEMOGLOBIN, CATTLE FIBRIN, CHICKEN EGG ALBUMIN AND SILK FIBROIN*

AMINO ACID	NUMBER OF AMINO ACID RESIDUES PER MOLECULE			
	Cattle Hemoglobin	Cattle Fibrin	Chicken Egg Albumin	Silk Fibroin
amino acids	$2^6 \times 3^2$	$2^6 \times 3^2$	$2^5 \times 2^2$	$2^5 \times 3^4$
ginine	$2^2 \times 3^1$	$2^5 \times 3^0$	$2^2 \times 3^1$	$2^2 \times 3^1$
sine	$2^2 \times 3^2$	$2^4 \times 2^1$	$2^2 \times 3^1$	$2^2 \times 3^0$
stidine	$2^5 \times 3^0$	$2^2 \times 3^1$	$2^2 \times 3^0$	$2^0 \times 3^0$
partic acid	$2^5 \times 3^0$	$2^5 \times 3^0$	$2^4 \times 3^0$	...
utamic acid	$2^4 \times 3^0$	$2^3 \times 3^2$	$2^2 \times 3^2$	...
ycine	...	...	...	$2^4 \times 3^4$
anine	...	...	...	$2^3 \times 3^4$
rosine	$2^2 \times 3^1$	...	$2^3 \times 3^0$	$2^1 \times 3^4$
oline	$2^2 \times 3^1$	$2^5 \times 3^3$	...	...
ypt'ophane	...	$2^1 \times 3^2$	...	...
steine	$2^0 \times 3^1$	$2^0 \times 3^2$	$2^2 \times 3^0$	...
ethionine	...	$2^2 \times 3^1$	$2^2 \times 3^1$	...

* Bergmann and Niemann (l.c.).

In the case of cattle hemoglobin the molecule contains 576 or $x3^2$ amino acid residues. Of this total there are 12 arginine, 32 tidine, 36 lysine, 32 aspartic acid, 16 glutamic acid, 12 tyrosine, 12 oline, and 3 cystine residues.

They show that the frequencies of the individual amino acid idues $\left(F_i \frac{N_t}{N_i}\right)$ that are contained in a molecule of protein can be pressed by the following equations:

(1) $N_t = 2^n \times 3^m$ where n and m are positive whole numbers.
(2) $N_i = 2^{n'} \times 3^{m'}$ where n′ and m′ are either zero or positive whole numbers.
(3) $F_i = 2^{n''} \times 3^{m''}$ where n″ and m″ are either zero or positive whole numbers.
(4) $n = n' + n''$, and $m = m' + m''$.
(5) $N_t = N_i' + N_i'' + N_i''' + \cdots\cdots\cdots\cdots N_i^x$.

N_t = total number of individual amino acid residues.
N_i = number of the individual amino acid residues.

It is significant that the experimentally determined values of N_i', $''$, N_i''', etc., and of F_i', F_i'', F_i''', etc. have led to values of N_t at are whole number multiples of 288 or $2^5 \times 3^2$, and it appears at the molecules of many proteins which do not show reversible

dissociation contain 288 amino acid units or a whole multiple th
Since this stoichiometrical law is of general validity it follow
every amino acid residue in the peptide chain of the protein mo
recurs at constant intervals. As an example, from the deductio
Bergmann and Niemann ('37), each glycine residue, G, in
fibroin is separated from the adjacent glycine residue by an a
acid residue other than glycine, e. g.,

–G–X–G–X–G–X–G–X–G–X–G–X–G–X–G–X–

Each residue of alanine, A, is separated from the adjacent
dues of alanine by 3 other residues, e. g.,

–A–X–X–X–A–X–X–X–A–X–X–X–A–X–X–X–A–X–X–X–

Each tyrosine residue, T, is separated from the adjacent tyr
residues by 15 other residues, e. g.,

–X–T–X–X–X–X–X–X–X–X–X–X–X–X–X–X–T–X–X–
X–X–X–X–X–X–X–X–X–X–X–X–X–T

On combining the above configurations the structure of a segr
of the silk fibroin molecule is obtained, i. e.,

G–A–G–X–G–A–G–X–G–A–G–T–G–A–G–X–G–A–G–X–
G–A–G–X–G–A–G–

To the older demonstrated fact that the linkage of the amino
residues is through the medium of the peptide bond (—CO—N
CHR—), to form long polypeptide chains, are added the new
cepts: (1) that the amino acid residues recur in a characteristic
periodic manner throughout the entire peptide chain, and (2)
these long chains fold upon themselves to form cyclic patterns, w
are held in a fabric structure. The latter, in turn, is folded into sp
enclosing geometrical figures (Wrinch). The physico-chemical
physiological properties of a protein are based on the frequen
with which its constituent amino acid residues recur within
peptide chains. Since many of the amino acids contain radicals ha
very different properties, e. g., the hydroxyphenyl group in tyro
the guanidine complex in arginine, the pyrrole nucleus in pro
and hydroxyproline, the —SH group in cystine, —OH group
serine and in hydroxyglutamic acid, and the indole nucleus in try
phane; and since, with a single exception (glycine), each of the an
acids contains an asymmetric carbon atom, and only one of the op
isomers of each plays a biological rôle, the fabric pattern, conce
of as representing the structure of the molecule of protein, mus

sualized as having various chemically active groups oriented in ace on either side of its median plane.

Monomolecular Films.—A new technic which offers extraordinary ssibilities for studying proteins has been described by Blodgett 35) and by Langmuir, Schaefer and Wrinch ('37). By means of odgett's methods, which cannot be described here, monomolecular ms, or multi-layer films of protein molecules, can be applied to a id surface such as chromium-plated brass plate. The plate is first ated with many layers of monofilms of barium stearate. A mono-olecular film of the protein (egg albumin or pepsin) is spread upon e surface of a tray of distilled water. There is then an upper, pro-n-air surface and an under, protein-water surface. If the plate is vered vertically through the film the latter becomes applied to its rface, the protein-air surface being next the barium stearate sur-ce. While the plate is submerged the protein layer is removed from e surface of the tray, and the plate then lifted out. It comes out t, whereas if the protein film had not been placed on the barium arate surface it would have come out dry, since the stearate surface es not wet with water. After evaporating the surface film of water e protein film of one molecule thickness is attached to the plate. amination of the surface with polarized light, at angles near the azing incidence, shows that the color differs markedly from the ginal barium stearate film. The thickness of the protein film is about

$$\frac{20}{0{,}000{,}000} \text{ cm.}$$

If, on the other hand, the plate is lowered into the water, and a nomolecular layer of protein is then spread upon the water sur-e, and the plate then lifted out, edge up, a protein film is applied its surface, but in this case it is the protein-water surface of the film ich is adjacent to the barium stearate surface. Again the plate mes out wet, and after evaporating the water the protein film comes o contact with the barium stearate. The film prepared in this way is led a B-film. By applying a film, drying the plate, and repeating dipping process, a second monofilm may be applied over the first. odgett designates the plate surface as P, the barium stearate surface R, and the protein films prepared by the two methods, as A film d B film respectively.

When a PRA system has been prepared and the plate is dipped o water having a monomolecular film of protein on its surface, a B n is applied giving a system PRAB. When this is dried and is vered into pure water or water having a protein film on its surface B film is ejected. If zinc chloride is added to the water the B film not ejected on dipping the plate, and AB layers can be applied ccessively, giving a system PRABABAB ..... indefinitely.

Without adding zinc salts to the water a succession of B fil be applied, giving a system PRBBB.....indefinitely. After a B film has been applied, and dried, the plate takes on an A film it is lowered through a protein film, giving PRBA, and on th film can be applied, giving PRBAB. In order to apply more la is necessary to add zinc salt.

A B layer which lies upon an A layer is ejected when the dipped, whereas a B layer which lies upon a B layer is not e From the method of applying the films it is evident that A laye inverted, or turned upside down in being applied, whereas layers are not inverted. Since the protein molecules are free to themselves when spread upon water, the hydrophylic groups o molecules orient to the water surface, and the hydrophobic g orient toward the air. The fact that A and B layers preserve identity after immersing in water indicates that they cannot re turn over. This, it is pointed out, supports the theory that the consist of a two-dimensional network rather than polypeptide ch "The most striking evidence that the outer surface of A and inner surface of B are predominantly hydrophylic is furnished b ejection on to the water of a B layer, which rests upon an A This action is undoubtedly caused by the affinity of this hydrop interface for water."

The employment of the supercentrifuge for the separation of teins by sedimentation, and the perfection of a technic for crystalli proteins, by Stanley, Wyckoff, and others, together with the met mentioned in this chapter, gives promise of steady advances in knowledge of these substances and the rôles which they play in ph logical processes. There is good reason to expect useful advance the discovery of special reagents for the quantitative determina of the amino acid content of proteins, and it is highly probable within a few years the composition and structure of many of the r readily obtainable proteins will be known with considerable exact The known rôles of proteins as enzymes, and as viruses, gre heighten the interest of investigators in this field. Further deve ments along these lines no doubt will be of use in the field of n tion.

Classification of the Proteins on the Basis of Their Chem Composition.—The classification of most proteins has been b upon their solubilities and other physical properties. Certain ones easily distinguished by their characteristic prosthetic groups, e mucoproteins, phosphoproteins, nucleoproteins, hemoglobins, etc. difficulty of perfecting analytical procedures for the accurate esti tion of amino acids delayed the accumulation of sufficient data serve as a basis of classifying proteins according to their amino

ntent. Block and Vickery ('31) made analyses of a number of the ghly insoluble proteins, usually grouped together as keratins, in hich they determined the content of the basic amino acids, arginine, stidine, and lysine, and the sulfur-containing amino acid cystine. heir data show that the keratin of human hair, sheep's wool, snake idermis, goose feathers, *Gorgonia flabellum* (the horny skeleton of orgonian coral), *Plexaurella dichotoma* (a related species of coral), d silk fibroin, all yield histidine, lysine, and arginine in such quanti- s that the molecular ratios of these amino acids are respectively proximately as 1:4:12. The cystine content varied greatly, being in e order given above: 16.5, 10.0, 5.3, 6.4, 5.5, 3.2 and 0.0. Block ('33a) nfirmed these ratios for the basic amino acids in keratin from ger nails and cattle horn.

The older view that the albumins and globulins of blood serum nstitute individual proteins has been disproved by Block ('33b), who owed that when the total protein of the serum of cattle is frac- nally precipitated by addition of different amounts of magnesium lfate or of ammonium sulfate, the various protein fractions obtained not have the same chemical composition based upon the determina- n of arginine, histidine, and lysine. The more soluble the protein solutions of these neutral salts, the greater was the content of ine. This seems to prove that the serum proteins are constructed a large number of non-dissociable components. The proteins ob- ned by the usual physiochemical methods are not of constant amino d composition. They are composed of two or more unstable co- ecipitation systems in mutual equilibrium. The amino acid com- sition of the total coagulable serum protein is, however, constant respect to the basic amino acids. Block ('34a) proposed the term *osin* to designate the total coagulable protein of the serum.

Block, Darrow and Cary ('33) determined the basic amino acids the serum of a patient suffering from nephrosis, in which the total otein (orosin) was 3.35 per cent as against the normal value of 7.0 cent. The molecular ratio of arginine to lysine was in both cases 17. Normal blood serum contains about 4.3 per cent of "albumin" d 2.7 per cent of "globulin." The serum derived from the nephrotic ient contained but 0.44 per cent of albumin and 2.91 per cent of bulin. The urinary protein in nephrosis resembles serum protein that the ratio of arginine to lysine is 10:18. Block et al. point out fallacy of the interpretation of clinical results in terms of the nged ratio of albumin to globulin. The basic amino acid content the orosins from human, dog and cow is the same. The work of se investigators emphasizes the fact that protein fractions derived m tissues, even by the mildest chemical procedures, are not neces- ily present as such in those tissues during life.

Block ('34a, l.c.) determined the basic amino acids in the
of hen, turkey, and duck. The molecular ratio of arginine to
was, in all cases, 10:11. He states that the average ratio b
these amino acids in 8 mammalian orosins is 10:18, and finds th
the more soluble protein fraction of serum protein (albumin
more soluble protein fraction of egg white protein contains a
content of lysine than does the less soluble fraction (globulin)
proportions of the basic amino acids yielded by hen orosin, a
levitin, the non-vitellin protein fraction of egg yolk, are the
From these studies it appears that the protoplasm of a specif
ganism, tissue, or cell mass, or certain derivatives thereof, is
posed of a labile nitrogenous chemical aggregate, tissue protein
yields arginine, histidine, and lysine in molecular ratios tha
approximately fixed, and are characteristic for the organism or
as it exists in various classes of animals. This observation stresse
primary importance of the basic amino acids in the genetic
embryological development of tissue protein.

Vickery and White ('33) found the cystine content of cryst
hemoglobins from horse, sheep, and dog to be 0.41, 0.61, and
per cent respectively, and the sulfur content 0.39, 0.73, and 0.5
cent respectively. The iron content of the three kinds of hemogl
was identical, viz., 0.33 per cent. The nitrogen content was
16.83, and 16.48 per cent respectively. The atomic ratios of
hemoglobins was, in the order named, 2.05, 4.00, and 3.02. The cy
content of the hemoglobins from three species of mammals is sh
to be quite distinctive, and this amino acid is in a different statu
compared with the basic ones, arginine, histidine, and lysine. Vic
and White suggest that the yields of other amino acids from h
globins of different origin may likewise vary widely.

Block ('34b, l.c.) has extended the study of the compositio
the hemoglobin of horse, sheep, and dog. He found that they
iron, arginine, histidine, and lysine in the molecular ratios of
proximately 1:3:8:9; while the molecular ratios of iron, sulfur, and
tine were 25:50:7, 25:100:14, and 25:75:21 respectively. He thus
firms the constancy of the molecular ratios of the basic amino a
and the content of iron, in these crystalline hemoglobins. The cy
content, on the other hand, varies over 300 per cent among them.

Synthesis of Proteins in Living Matter.—The idea that enzyn
action, which is manifest through hydrolysis in the alimentary t
might be reversible, and that proteolytic enzymes might be able, w
in the presence of a concentrated system of digestion products
synthesize protein, is not new. It was first put to experimental te
1886 by Danilewski, who observed the formation of a precipitat

rotein when an extract of stomach containing pepsin was added to concentrated solution of the products of peptic hydrolysis. Others ave reported the synthesis of protein by means of trypsin and pepsin. he history of these investigations is reviewed by Wasteneys and orsook ('30) who have defined the conditions under which the naximum amount of synthesis of protein-like material known as plastein" is formed. They showed that it is of protein nature. Levene nd Van Slyke ('08) carried out an amino acid analysis of plastein, and ound it to contain at least 13 amino acids. Beard ('26) demonstrated y feeding experiments that plastein prepared from digestion products f egg white satisfies the nitrogenous needs of growing mice. It is bvious that an understanding of the minute structure of these synetic proteins of the plastein type, and a comparison of them with ne structure of the proteins from which they were derived, after igestion, is not possible until the amino acids can all be determined uantitatively, and until some method is worked out for determining ne order of arrangement of the amino acid residues in the peptide nains which constitute the protein molecules. Distinct progress has een made in both directions.

Alcock ('36) has also discussed plastein formation, and expresses ne belief that "it is probably safer to describe the reaction as peptone olymerization than as a synthesis of protein." This view is difficult o harmonize with the observation of Henriques and Gjaldbäk ('11), ho furnished unequivocal evidence that there is a decrease in the ee amino nitrogen in a digest after plastein formation. As a protein digested (hydrolyzed) the amount of free amino nitrogen steadily ncreases. A decrease must be accepted as evidence of anhydride formaon, i. e., formation of peptides. When plastein is digested by pepsin, ne free amino nitrogen in the system increases just as is the case with rotein.

The view that proteins are synthesized by the piecing together of eir constituent amino acids, with multiple anhydride formation beveen each pair, would appear to rest upon convincing evidence, pecially that of Cary and Meigs ('28). In the cow, while secreting ilk, the concentration of amino acids in the blood of the vessels fferent to the gland is greater than in the efferent. It is assumed at casein, or other milk protein, is formed from the amino acids hich have disappeared during passage of blood through the gland, nce they are not present as such in the milk. If this is not the source the casein, from where does it come?

Notwithstanding the general acceptance, by biochemists, of the nthesis of proteins from amino acids in living tissues by putting em together into chains by anhydride formation, this view is re-

jected by Alcock, who suggests an alternative origin of th
enchymatous and tissue proteins. He postulates that the anima
with something simpler than the amino acids derived by str
down the molecules of the essential ones, and the polymeriza
this substance, or substances, into a homogeneous molecule.
he believes, undergo a process of internal differentiation during
the amino acids are formed. He points out that at no stage in
synthesis in the animal is there a high concentration of the
intermediates, the polypeptides, as evidence against the theory
stepwise building of proteins—a view which has never been
pioned. He conceives the rôles of the non-essential and essential
acids in a new light, illustrating the methods by which the body
with a deficiency of the members of each class, using glutamic
(dispensable), and tryptophane (indispensable), as examples
writes, "It is necessary to picture the state of affairs in an a
living on a diet lacking in some one amino acid; say glutamic
During the shortage of glutamic acid the animal continues to
up proteins (and glutathione); continues, in fact, to lay down glu
acid, and it is possible to maintain an animal in this way so
that it may lose appreciable quantities of the amino-acid in its
hair and skin. Where does the glutamic acid come from? There
two possibilities. It may be synthesized in some special organ
the liver, or it may on the other hand be produced locally at the
of protein synthesis. If the former, then it would seem that
mechanism exists, compensating the shortage of glutamic acid
specific synthesis. The same must apply for all other dispens
amino acids. The possibility, which implies an extremely com
mechanism, which is only called into play under the most abno
conditions, must be dismissed as highly unlikely."

In the case of deficiency of an essential amino acid, say try
phane, "Deprival results in a syndrome resembling that followi
vitamin lack; in particular, there is failure to grow, or even a
nite loss of weight." The orthodox view is that limitation to pro
synthesis is imposed by lack of tryptophane. Alcock suggests
tryptophane has some other function which accounts for its i
pensability, and that the observed adverse effects of its defici
are due to this function. He points out that Berg and Rose
have shown that the demand for tryptophane is immediate,
feeding large doses at relatively long intervals is not equivalen
feeding smaller doses at shorter intervals. If a rat is starved
tryptophane for 24 hours it shows the effects. Allusion is mad
the reports that when tryptophane is injected into an anemic ani
the return of the blood to normal composition is accelerated;
that its injection into a normal animal causes hyperemia; but

wn observations show that recovery from anemia is possible with-
ut tryptophane. Alcock believes that while tryptophane has some
finite function other than as a component of the protein mole-
ule, it is still a mystery. In animals without tryptophane, he reports
rolonged survival with some blood formation, hair, and skin growth,
nd secretion of digestive fluids containing protein (and tryptophane).
hese observations are cited as evidence that the failure of animals
put on weight in tryptophane deficiency is definitely to be referred
the interference with the "essential" function of that amino acid,
nd that protein synthesis (and tryptophane synthesis) would go on
nimpaired if that function could be restored. Alcock has observed
animals deprived of tryptophane, and injected with anterior pitui-
ary growth hormone, sufficient increase in weight to indicate growth
nd protein synthesis. The other essential amino acids are, like trypto-
hane, believed to owe their indispensability, not to inability of the
ody to synthesize them in the process of protein synthesis, but to
heir "essential" functions.

The crucial part of the theory is nicely summarized by Du-
igneaud, Sealock and Van Etten ('36), who state: "Alcock sug-
ested that the limiting factor to growth on a tryptophane-deficient
iet does not arise from the demand of the tissues for amino acids
be used for protein synthesis but that some material essential to
fe was made in the liver from tryptophane absorbed from the in-
stinal tract, whereas tryptophane delivered elsewhere than in the
ortal vein was not available for the 'essential' purpose. He also con-
luded that the animal can synthesize its proteins while receiving no
yptophane and that for this particular purpose the body can make
s own tryptophane. He further pointed out that this conclusion
nplied that the synthesis of a protein probably starts at a lower
age than the fully formed amino acid and that the amino acid
roups actually found in the proteins have been formed there, not
ssimilated from the blood stream as such."

Alcock, therefore, rejects the idea that proteins are synthesized
y an enzymatic condensation of amino acids. Instead he offers the
uggestion that a common attribute of living creatures is the synthesis
f a primitive type of protein from relatively simple materials. The
lood amino acids are taken up by the tissues and broken down to
me simple unit. This unit, by polymerization, provides a basis on
hich the protein is built. Within this molecule of primitive protein,
r "urprotein," a process of differentiation sets in and a definite pro-
ortion of each amino acid is formed, the protein so produced being
milar in composition to the probable ancestral protein. The subse-
uent differentiation of the molecule, involving addition or elimina-
on of amino acids, or the addition of prosthetic groups, follows a

course determined by the nature of the tissue in which it is produced.

A cogent argument against Alcock's theory of protein sy would seem to be the behavior of the animal in fasting. It i known that during fasting the muscle proteins undergo autol an extent sufficient to maintain the amino acid content of the at a constant fasting level. The orthodox view that this mech serves to maintain an adequate nutrient medium, as respects acids, for the nutrition of the organs and nervous system, and permit survival of the organism for a time at the expense o more dispensable tissues, seems to argue convincingly agains possibility that all amino acids can be synthesized by the tissue

Moreover, DuVigneaud, Sealock and Van Etten (l.c.) hav cently reported that tryptophane injected subcutaneously ca utilized. Since the decisive point in Alcock's theory is his failu obtain growth of rats injected with tryptophane on a tryptop deficient diet it is evident that the theory, although interesting provocative, is based upon very questionable evidence.

Rôle of Enzymes as Specific Organizers in Protein Synthes

In discussing plastein formation it was pointed out that proteo enzymes appear to be able to produce polypeptides from amino a by anhydride formation. This view is opposed to the older con that the only function of these enzymes is the hydrolytic degr tion of proteins into polypeptides, dipeptides, and amino acids. obvious that some mechanism exists for the synthesis of protein the body, for it is well established that proteins are degraded in digestive tract into amino acids and perhaps some simple pepti which enter the blood and serve as the pabulum for protein nu tion. Bergmann and Niemann (l.c.) conceive that since protein m cules are assembled from hundreds or thousands of amino acids cording to an unvarying pattern, the conclusion is unavoidable these gigantic molecules are synthesized under the control of s specific organizer. This organizer must, of course, be able to ind anhydride formation to produce the peptide bond (—CO—NH CHR—). It must also be able to select, from all the available am acids and peptides, a particular amino acid or peptide at the cor moment, so that the unequivocal pattern of the complete pro molecule is adhered to in every stage of the synthesis. This invo the promotion of hundreds of consecutive reactions, each highly cific, and each of which alters the structure of the substrate thereby the character of the subsequent specific reactions. They d it likely that the intracellular proteinases are in reality the pro organizers. They state: "The intracellular enzymes are able to grade proteins through hydrolysis of the peptide bond, and recentl

as been demonstrated that this splitting proceeds so far that lower olecular weight peptides and even amino acids are formed. On the her hand, these same intracellular enzymes are capable of promotg the synthesis of the peptide bond, thereby forming higher order eptides from the lower molecular weight protein hydrolysis prodcts. It appears that the reason that the intracellular enzymes are able promote hydrolysis and synthesis under the same experimental onditions, with the result that these two reactions are in competion with each other, is that small differences in the structure of the ibstrate coupled with the very exactly tuned specificity of the indidual intracellular enzymes determines whether or not and to what egree synthesis or hydrolysis shall occur. The organizing ability of he individual intracellular proteinases is concomitant with the possession of the above properties."

An interesting example of the synthetic action of an enzyme is rought to light by Bergmann and Fraenkel-Conrat (Bergmann and Niemann, l.c.). The enzyme papain acts upon two very similar pepde-like compounds, benzoylglycine amide and benzoylglycine anilide. enzoylglycine anilide is not only hydrolyzed by the enzyme, but is ynthesized by the enzyme in the presence of benzoylglycine and niline:

$$C_6H_5CONHCH_2CONH_2 \xrightarrow{\text{enzymatic hydrolysis}} C_6H_5CONHCH_2COOH + NH_3$$

$$C_6H_5CONHCH_2CONHC_6H_5 \xleftarrow{\text{enzymatic synthesis}} C_6H_5CONHCH_2COOH + C_6H_5NH_2$$

In this "model" Bergmann points out that the amide plays the ôle of the extracellular proteins, and the anilide that of the intraellular proteins. Bergmann and Niemann suggest that since the roteolytic enzymes are themselves proteins (Sumner, Northrop), hen there must exist other proteinases which have the ability to ynthesize replicas of their own structural pattern and are able, therore, to "multiply" in suitable surroundings. The tobacco mosaic virus f Stanley, which is a protein with an extraordinarily high molecular veight, would seem to be an example of this property. When placed in he nutrient medium afforded by the host organism, it would utilize he circulating amino acids, and induce the continuous production f its replica, a foreign protein. This process would account for the nown behavior of viruses and the normal growth of animals using mino acids from entirely different proteins.

Amino Acid Composition of Tissue in Relation to Composition of Dietary Proteins.—Owing to the apparently complete degradation

of dietary protein to amino acids, or very simple peptides, befo
synthesis of such nutrient principles into tissue proteins, there i
siderable reason to doubt that the amino acid composition of
proteins is influenced by dietary factors. However, some investig
particularly Schenck and Wollschnitt ('33), claim, for example,
the amounts of tryptophane in liver, kidney, and muscle prote
rats is influenced by the composition of the dietary protein. The
tion has been studied by Lee and Lewis ('34), who report essen
opposite conclusions. It is significant, however, that in their young
receiving an adequate supply of dietary cystine there was a hi
content of sulfur in the tissues than in similar rats receiving a
deficient in cystine. This is attributed, with justification, to the
ence of larger amounts of non-protein sulfur in animals inges
the cystine-adequate diet.

The effect of diet on the protein or amino acid content of
and eggs is also of interest. There seems to be a very inadeq
amount of evidence regarding milk, and it is not yet clear whe
the composition of egg proteins is affected by diet. For example,
differences could be found in the composition of proteins of eggs f
white Leghorn pullets raised and kept on diets having entirely
ferent sources of proteins, namely, wheat middlings, ground c
corn gluten, and soy bean oil cake meal (Calvery and Titus, '
Even the hatchability of eggs, as influenced by diet, apparently is
related to the content of total nitrogen, total amino nitrogen, tyrosi
tryptophane, and cystine (McFarlane, Fulmer and Jukes, '30). E
the recent report of Patton and Palmer ('36) is of special interest
this regard. Although they found that "optimum" versus "deficie
protein rations caused no significant changes in the tryptophane, ty
sine, histidine, arginine, cystine, lysine, and glycine of egg prote
there was a significant difference between the glycine contents
normal and chondrodystrophic chick embryos. Since the content
glycine in foodstuffs seems to bear some relation to their efficacy
preventing the occurrence of chondrodystrophic embryos it may
tentatively assumed that the amino acid composition of dietary prot
does have some influence on the amino acid metabolism of eggs d
ing embryonic development. It may be concluded that further stu
is definitely needed. But, as suggested in the discussion of organiz
in protein synthesis, there is scarcely any basis for the assumption t
protein factors in the diet may influence the composition of tissues

***Amino Acid Composition of Protein in Relation to its Nutriti
Value.***—Kossel, Fischer, and Osborne, pioneers in protein analy
made it clear that very pronounced differences exist in the compo
tion of proteins from various sources. Although their technics w

ever perfected so as to give results approximately quantitative, except the case of less than a third of the known amino acids, it was nown in these few cases that there are great variations in the proortions of amino acids obtained from proteins from different ources. Thus, the proteins of the muscle tissues of several species of imals yield between 12 and 14 per cent of glutamic acid. This amino id is present in the two principal proteins of the wheat kernel to e extent of about 40 per cent. Other equally great differences were own to exist in the composition of proteins of common foods.

Since nine or more of the amino acids are, apparently, totally inpable of being synthesized by the body, it is necessary that they provided in the diet in such amounts as are essential to the various dily structures and functions. It is obvious, therefore, that the actical problem in protein nutrition is the provision of foodstuffs nose proteins furnish amino acids needed by the body, and in the quisite amounts. In the present state of our knowledge it appears at the problem may be illustrated by comparing the digestion oducts of the protein molecule to the letters of the alphabet. he proteins of the food and of the body tissues, which are unlike each her, may be regarded as made up of the same letters arranged in different orders and present in different proportions. During growth the gested food proteins are resolved into the constituent amino acids and haps some simple peptides. These, after absorption, are reassembled new order, and in new proportions to form the tissue proteins and mote bodily functions. For example, let the muscle tissue of an anil be compared to a block of printer's type so arranged as to print the me "Jack Spratt could eat no fat, and his wife could eat no lean." en allow the proteins of which the muscle consists to be represented the individual words, and the protein digestion products, i. e., ino acids, by the letters of which the words consist. Now let animal ingest food proteins which correspond to a block of type t would print the jingle "Peter Piper picked a peck of pickled ppers." It is easy to understand that when the proteins of the d are resolved into their constituent letters, namely, amino acids, an effort is made to form the bodily proteins of the new and erent printer's type from the letters supplied by the food, the nsformation cannot be made.

The significance of this illustration is shown concretely by the ritive behavior of the protein of corn, zein, and the amino acids, ne and tryptophane, in which zein is particularly deficient. Muscle ue protein contains considerable amounts of lysine and tryptone. If these should correspond to the Jack Spratt jingle and should correspond to the Peter Piper jingle it is obvious that

lysine and tryptophane (Jack Spratt) could not be furnish muscle tissue growth since zein (Peter Piper) does not supply amino acids.

It is thus of importance in nutrition to consider the actual acid composition of proteins and protein-containing foods sin nutritional value of such foods is absolutely dependent upon course such factors as digestibility of protein, absorbability of acids, and ratio of absorbed amino acids to body amino acid re ments, must be considered in the nutritional evaluation of ch data on proteins and protein-foods. Since these factors are only tainable by means of biological assay it is not sufficient to only the amino acid content of such foods. Nevertheless such obviously are of practical value and it is well to consider some of the evidence regarding this subject.

There is considerable variation in the amino acid content of tially similar foodstuffs as is indicated by Csonka's ('37) dat the amino acid content of different wheat varieties. This is sh in the somewhat modified table taken from his paper.

PROTEIN QUALITY AS SHOWN BY RELATIONSHIP BETWEEN AMINO ACIDS AND TOTAL NITROGEN CONTENT

VARIETY	MG. OF INDICATED AMINO ACID PER GM. O TOTAL NITROGEN					
	Cystine	Tryptophane	Tyrosine	Arginine	Histidine	Ly
Marquis	81	28	262	154	85	4
Tenmarq	65	26	173	146	43	4
Fulhio	67	34	217	152	34	3
Casein (for comparison)	20	130	405	236	156	4

Cereal proteins in general contain considerably less lysine tryptophane than do animal proteins. This is indicated for try phane in the above table but in this case no very significant di ence seems to exist between the lysine content of wheat and ca As a rule cereal and vegetable proteins are relatively deficien lysine. But, the real significance of this table is the marked va differences in the composition of proteins. Hence it should be re nized that tables on protein composition provide only a general dication of the actual amino acid content of a given protein or pro food.

A useful amount of data on the amino acid composition of the principal purified proteins is furnished by the table that follows:

AMOUNTS OF AMINO ACIDS ISOLATED FROM PROTEINS

	Ovalbumin	Lactalbumin	Casein	Vitellin	Hemoglobin	Gelatin	Silk Fibroin	Wool Keratin	Salmin	Wheat Gliadin	Wheat Glutenin	Edestin (Hemp)	Legumin (Pea)	Amandin (Almond)
lycine	0.0	0.0	0.0	0.0	..	25.3	36.0	0.6	...	0.0	0.9	3.8	0.4	0.5
anine	8.4	2.5	1.5	0.8	4.2	8.7	21.0	4.4	..	2.0	4.7	3.6	2.1	1.4
line	..	0.9	7.2	1.9	..	0.0	0.0	2.8	4.3	3.4	0.2	..	..	0.2
ucine and iso-leucine	15.2	19.4	9.4	9.9	29.0	7.1	1.5	11.5	..	6.6	6.0	20.9	8.0	4.5
partic acid	6.1	1.0	4.1	2.2	4.4	3.4	..	2.3	..	0.6	0.9	4.5	5.3	5.4
utamic acid	14.0	10.1	21.6	13.0	1.7	5.8	0.0	12.9	..	43.7	23.4	6.3	17.0	23.2
rine	..	..	0.5	..	0.6	0.4	1.6	0.1	7.8	0.2	0.7	0.3	0.5	..
ydroxyglutamic acid	1.4	..	10.5	..	..	0.0	..	..	..	..	..	..	..	..
enylalanine	5.2	2.4	3.2	2.6	4.2	1.4	1.5	0.0	..	2.4	2.0	2.4	3.8	2.5
rosine	3.2	0.9	4.5	3.4	1.3	0.0	10.5	2.9	..	1.2	4.3	2.1	1.6	1.1
yptophane	..	..	1.7	..	..	..	..	..	..	1.0	..	..	..	..
stine	0.4	..	..	..	0.3	..	..	7.3	..	0.5	0.02	..	..	..
thionine	2.5	..	1.4	..	..	..	..	..	..	..	..	..	..	..
sine	5.0	9.2	6.0	5.4	8.1	5.9	0.3	2.3	0.0	0.2	1.9	2.2	5.0	0.7
inine	5.4	3.2	3.8	7.9	3.3	9.1	0.7	7.8	87.4	3.2	4.7	15.8	11.7	11.9
stidine	1.4	2.1	2.5	1.2	7.6	0.9	0.1	0.7	0.0	0.6	1.8	2.1	2.4	1.6
line	4.2	4.0	8.0	4.2	2.3	19.7	0.3	4.4	11.0	13.2	4.2	1.7	3.2	2.5
droxyproline	..	..	0.3	..	1.0	14.4	..	..	..	..	..	2.0	..	..
mmonia	..	1.3	1.6	1.3	..	0.4	..	..	..	5.2	4.0	..	2.1	3.7
Total	72.4	57.0	87.8	53.8	68.0	102.1	73.5	60.0	110.5	84.0	59.72	61.4	63.1	59.2

Plimmer in Harrow & Sherwin, *Biochemistry*. Courtesy, W. B. Saunders Company.

It will be observed that gelatin and zein are especially deficient certain amino acids. This chemical evidence is supported by the arked failure of these proteins to promote growth or even main-nance unless supplemented with the missing essentials, either as otein or the free amino acids. Edestin of hemp seed is noteworthy that it contains sufficient amounts of all the indispensable amino ds to promote growth. It is somewhat unique in this respect since ost of the vegetable and cereal proteins appear to be deficient in e or more of the essential amino acids. The known high nutritive ue of casein and lactalbumin is indicated by their amino acid mposition.

The following table furnishes more recent information on the ntent of certain amino acids in some proteins and protein-containing ds of general importance. In the table no distinction is made be-een values based on colorimetric procedures and those obtained isolation of the amino acids. Also, no distinction is made between tine and cysteine in the cystine column.

THE CONTENT OF CERTAIN AMINO ACIDS IN PROTEINS AND PROTEIN-CONTAINING FOODS

PROTEIN OR PROTEIN-CONTAINING FOOD	INVESTIGATION	PER CENT OF THE AMINO ACIDS								
		CYSTINE	METHIONINE	TRYPTOPHANE	TYROSINE	PHENYLALANINE	ARGININE	HISTIDINE	LYSINE	GLUTAMIC ACID
Casein	(1)	..	0.41	..	..	..	..	..	..	.
Casein	(2)	..	..	..	..	..	3.85	1.83	6.25	.
Highly purified egg albumin	(3)	1.33	..	1.28	4.21	..	5.03	2.44	6.41	13.9
Vitellin of hen's egg	(4)	1.19	..	1.24	5.01	..	7.77	1.22	5.38	.
Thyroglobulin	(5)	..	..	..	..	..	8.22	0.62	1.93	.
Globulin of sunflower seed	(6)	..	..	..	..	..	9.10	14.29	1.80	.
Protein of soy bean	(7)	..	..	..	3.82	5.21	..	..	..	.
" " " "	(1)	..	0.08	..	..	..	..	..	..	.
Protein of Cocksfoot (forage grass)	(8)	..	..	..	..	..	1.36	1.66	5.27	.
Wheat bran	(9)	0.25	..	0.28	..	..	0.85	0.18	0.54	.
Wheat endosperm	(9)	0.14	..	0.12	..	..	0.36	0.20	0.23	.
Water-soluble baker's yeast protein by heat coagulation	(10)	0.44	..	2.66	4.79	..	..	..	..	..
Alkali-soluble baker's yeast protein	(10)	0.69	..	1.79	3.11	..	..	..	..	..
Water-soluble brewer's yeast protein by heat coagulation	(10)	0.49	..	2.67	4.11	..	3.50	1.38	4.50	..
Alkali-soluble brewer's yeast protein	(10)	0.31	..	1.68	3.93	..	2.82	trace	4.53	..
Muscle protein of sardine	(1)	..	0.52	..	..	..	..	..	..	..
Muscle protein of fresh cod fish	(11)	0.6	0.3	2.1	2.0	1.1	6.8	..	..	7.5

References for table

(1) Tomijama, T., and Hanada, M. J. Biochem., *19*, 345–351, 1934.
(2) Vickery, H. B., and White, A. J. Biol. Chem., *103*, 413–415, 1933.
(3) Calvery, H. O. J. Biol. Chem., *94*, 613–634, 1932.
(4) Calvery, H. O., and White, A. J. Biol. Chem., *94*, 635–639, 1932.
(5) White, A., and Gordon, W. G. Proc. Soc. Exper. Biol. Med., *32*, 354–357, 1934.
(6) Blagoveshchenskii, A. V., and Schubert, T. A. Biochem. Ztschr., *269*, 375–378, 193
(7) Sasaki, S. J. Agric. Chem. Soc. (Japan), *11*, 321–330, 1935; Chem. Abstr., *29*, 6616,
(8) Miller, E. J. Biochem. J., *29*, 2344–2350, 1935.
(9) Murphy, J. C., and Jones, D. B. J. Biol. Chem., *69*, 85–99, 1926.
(10) Csonka, F. A. J. Biol. Chem., *109*, 703–715, 1935.
(11) Abderhalden, E., Baertich, E., and Ziesecke, W. Ztschr. f. physiol. Chem., *240*, 162, 1936.

The Dispensable and Indispensable Amino Acids in Nutritio

It has long been recognized that the only method of obtaining equivocal evidence of the role of individual amino acids in nutri is to observe the response of animals to diets in which the pro moiety is replaced entirely by mixtures of amino acids, the c position of which can be varied at will. In 1912 Abderhalden tempted such a study, using amino acid mixtures prepared by digestion of meat with appropriate enzymes. Dogs fed this diet m large growth gains but the removal of tyrosine and tryptophane fr the mixture caused marked failure in both growth and maintena Since 1912 various workers have made some useful contributions to knowledge but owing perhaps to the large cost of such investigati

elatively slight advances had been made until the recent work of V. C. Rose and his collaborators. The subject has been reviewed by Rose ('38).

Glycine. On the basis of various types of evidence it is concluded hat glycine is dispensable. Animals fed large amounts of benzoic cid excrete more glycine in the form of hippuric acid, a condensaion product of benzoic acid and glycine, than can be derived from he proteins catabolized. It is generally regarded that the synthesized lycine may be formed from ammonia and non-nitrogenous mateials, or from other amino acids. It should be stated, however, that recursors of glycine do not appear to be readily available in proein metabolism (Griffith, '34). Aside from other secondary indicaions of dispensability the crucial evidence was furnished by McCoy nd Rose ('37), who found that a glycine-free mixture of amino acids, ed with an appropriate basal diet, promoted good growth. The inlusion of glycine in the diet did not influence growth.

Alanine. There is no proof that alanine is essential. Rose's feedng experiments indicate dispensability, as do those of Abderhalden '22).

Serine. Like other hydroxyamino acids, i. e., hydroxyglutamic acid, ydroxyproline, and tyrosine, there is no certainty that serine is essenal. Definite indications of its dispensability were furnished by McCoy nd Rose (l.c.) simultaneously with their studies of glycine.

Valine. The only definite evidence of the role of this amino acid furnished by Rose ('37), who claims that it is indeed indispensable nce its absence from the stock amino acid mixture causes a proound failure in growth accompanied by a hypersensitiveness to touch nd a lack of coordination in movement. A study of the pathology of valine deficiency should be made since it appears probable hat the nervous system is directly affected by its absence from the et.

Leucine. Again the only worker to furnish very clear-cut infor- ation is Rose. Womack and Rose ('36), using the Rose procedure, und that leucine is decidedly an indispensable amino acid for rowth.

Isoleucine. As in the case of leucine, the only dependable data e those of Rose. Womack and Rose (l.c.) showed that growth canot continue in the absence of isoleucine. Therefore that amino acid essential. It should be noted that valine, leucine, and isoleucine ave one common structural similarity, namely, a secondary carbon nkage which is in either the beta or gamma position.

Norleucine. Apparently norleucine is not needed in the diet but nal conclusions must await further confirmation (Womack and ose, l.c.).

Aspartic acid. This amino acid appears to be dispensable as s by Bunney and Rose ('28). Likewise Hopkins ('16) obtained a ently successful nutrition in the absence of aspartic acid.

Glutamic acid. As in the case of aspartic acid, Bunney and found glutamic acid dispensable. Various studies of the detoxic of phenylacetic acid by means of glutamine, probably the only rally occurring form of glutamic acid, suggest the capacity o body to synthesize glutamic acid. Also, Hopkins' (l.c.) feedin periments with glutamic acid-low diets indicate its dispensabili

Hydroxyglutamic acid. Abderhalden's ('22, l.c.) early st pointed to its dispensability and the work of Windus, Catherw and Rose ('31) confirmed that conclusion.

Arginine. The status of arginine in nutrition has been in ques for many years. In 1916 Ackroyd and Hopkins reported that were able to grow when the arginine- and histidine-free pro hydrolysate, furnishing the dietary amino acids, was supplemer with arginine and histidine, either together or singly. It was cluded, therefore, that arginine and histidine are interconvertibl nutrition. The plausibility of this view is supported by the simila in structure of these two compounds, c. f. amino acid formulae. neither Abderhalden ('22, l.c.) nor Rose and Cox ('24) were to confirm the replaceability of histidine by arginine. Stewart's (results failed to confirm those of Ackroyd and Hopkins, as well those of Rose et al. Owing to present events, Stewart's observati probably are significant. Arnold and associates ('36), working w chicks, seem to have demonstrated that arginine is definitely in pensable for rapid growth. This work is of importance especially view of their basal diet. It was:

Dextrin	64	Crude liver extract	2
Purified casein	18	Autoclaved liver extract residue	2
Water-extracted lung	5	Brewer's yeast	2
Salts	5	Cod liver oil	2

Chicks on this basal ration weighed about 335 gm. at the end 6 weeks but when arginine was added at a level of 1 per cent weight was about 390 gm. after a similar period of 6 weeks. Th findings have been confirmed by Klose, Stokstad and Almquist ('3 who found that increasing the dietary casein to 30 per cent provid sufficient arginine for maximum growth. Heretofore it has been sumed with some justification that all the amino acids, with possible exception of cystine and methionine, are furnished in a quate amounts by a diet containing 18 per cent of casein. Arnold et have not yet submitted any evidence regarding the relation of argini to histidine in growth.

In view of the evidence from chick nutrition and the obser

ions of Scull and Rose ('30) that rats on an arginine-free diet grow t only about three-fourths of the normal rate—whereas addition of rginine to the food remedies the dietary defect—it must be concluded that arginine probably is synthesized in considerable amounts n the body, but during rapid growth an exogenous source of the mino acid must be furnished. Hence arginine may be tentatively egarded as indispensable because synthesis is too slow for rapid rowth.

Lysine. Although the necessity of lysine was conclusively demonstrated by Osborne and Mendel, and others, during the period of 914-1920, by means of its ability to induce growth when added to liadin rations or to zein rations supplemented with tryptophane, here has been no considerable attempt to augment those studies with urified amino acid mixtures. Abderhalden's ('22, l.c.) work with mino acid mixtures, since proved to be faulty in some respects, indiated the indispensability of lysine. According to Rose's ('37, l.c.) reliminary evidence the minimal amount of lysine necessary to support normal growth of rats is 1.0 per cent, i. e., the diet must contain .0 per cent of lysine. This value is higher than that for any other mino acid thus far reported. Since this amino acid tends to be low n vegetable proteins the above observation may be of more than rdinary practical significance.

There have been some studies of the replaceability of lysine in the iet by related hydroxy compounds. McGinty, Lewis and Marvel ('24) ound that natural l-lysine promoted somewhat better growth than id synthetic dl-lysine. This has been verified by Berg and Dalton '34), whose work indicates that the unnatural d-lysine of the dlixture could probably not be utilized at all for growth. Direct proof f this has been furnished by Berg ('36) and Rose ('37, l.c.). In this espect lysine is comparable to cystine but different from tryptophane, istidine, and methionine.

Histidine. Owing to its close structural similarity to arginine, as ndicated previously, the nutritive role of histidine has been studied, n part, with reference to its interchangeability in the body with rginine. The early work of Ackroyd and Hopkins ('16), which sugested such a relationship, became questionable when Rose and Cox '24, l.c.) showed that rats on an arginine and histidine-deficient diet ere unable to grow or even maintain body weight when only rginine was added. However, the addition of histidine alone, or ith arginine, caused resumption of growth. On the basis of this udy it has been definitely concluded that histidine is indispensable.

Histidine, however, is replaceable by certain closely related compounds, as was first demonstrated by Cox and Rose ('26) when it as shown that dl-β-4-imidazole lactic acid added to a histidine-defi-

cient diet caused resumption of growth. Amino-N-methyl histi also replaces histidine for growth of rats (Fishman and White, Finally, as shown by DuVigneaud, Sifferd and Irving ('37), l-ca sine (β-alanyl-l-histidine) can replace histidine. According to t authors, injected histidine can be utilized for growth purposes.

Tryptophane. The establishment of tryptophane as an esse dietary factor, by Willcock and Hopkins in 1906, constituted the definite evidence of the indispensability of an organic substance wh chemical nature was known at the time. Since then the various stu of Osborne and Mendel ('12), Abderhalden ('13), and Berg and R ('29, l.c.) have conclusively demonstrated its indispensability. In v of Alcock's (l.c.) radically different concept of protein metaboli based considerably upon the failure of Jackson ('27) and himself observe utilization of tryptophane injected subcutaneously, there been some interest in the utilization of this amino acid as affec by the route of intake. But DuVigneaud, Sealock and Van Ette (l.c.) conclusive evidence of the satisfactory utilization of tryptopha injected subcutaneously leaves Alcock's contention without basis. T fact that utilization of tryptophane is much more efficient if the jections are made at several intervals per 24 hours, as shown by Be and Rose ('29, l.c.) and DuVigneaud et al. ('36, l.c.), is to be expect since all substances behave that way.

Of particular interest to considerations of the intermediary metab lism of tryptophane is the work on compounds which replace th amino acid in growth. Those which function rather efficiently this regard are 3-indole-pyruvic acid (Jackson, '29; Berg, Rose a Marvel, '29), acetyl-l-tryptophane (DuVigneaud, Sealock and Va Etten, '32), and amino-N-methyltryptophane (Gordon and Jackso '35). But, whereas, optically unnatural d-tryptophane can be utilize for growth, the acetyl form, acetyl-d-tryptophane, is not utilized, contrast to the effect of acetyl-l-tryptophane (DuVigneaud, Sealoc and Van Etten). There is need of further investigation concernin the basis for these facts.

Thus far no definite evidence is available with respect to any sp cial nutritive functions which might be possessed by tryptophane. Th symptoms of its deprivation are failure of growth, loss of weigh loss of hair, hardening of the skin, and depletion of fat stores. Ther seems to be no conclusive evidence that tryptophane is the sourc of the pyrrole group of hematin.

Phenylalanine and tyrosine. The early studies of Abderhalden ('15 indicated that tyrosine is a nutritive essential but Totani's ('16) ob servations, which appeared to be more convincing, indicated th dispensability of that amino acid. Owing to the very close similarit in chemical configuration between phenylalanine and tyrosine it wa

arly suggested that they may be mutually interchangeable in metabo-sm. This idea was given considerable credence owing to the obser-ation of Embden and Baldes ('13) that the perfusion of the surviving iver with blood containing phenylalanine leads to the production f small amounts of tyrosine. But according to Shambaugh, Lewis nd Tourtellotte ('31), the two amino acids when administered in noderate doses, do not yield identical intermediates.

Apparently this question concerning these compounds has been nally answered. Womack and Rose (l.c.), by means of a mixture f amino acids free from phenylalanine and tyrosine, adduced con-incing evidence that phenylalanine is a growth requisite which can-ot be successfully replaced with tyrosine. Tyrosine deficiency alone ermitted good growth and its addition to the diet provided no indi-ation that this amino acid is needed. Therefore phenylalanine is ndispensable and tyrosine is dispensable.

Proline and hydroxyproline. In view of St. Julian and Rose's ('32) onvincing studies which demonstrate the dispensability of both pro-ne and hydroxyproline the older questionable evidence for the in-ispensability of proline (Sure, '24) is no longer tenable.

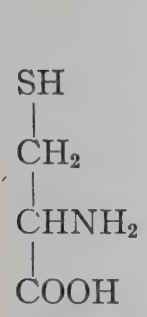
Threonine. It is generally recognized that the most laudable in-estigation in the modern era of protein nutrition is that of Rose nd associates concerning the discovery of evidence for, and the ventual isolation and identification of, a hitherto unrecognized and ndispensable amino acid, namely, α-amino-β-hydroxy-n-butyric acid. he common name assigned to it by Rose is d-threonine, inasmuch s its spatial configuration is exactly analogous to that of the sugar -threose. Space does not permit the extensive account of this work hat its importance deserves. Some of the original papers are: Rose 32), McCoy, Meyer and Rose ('35), and Meyer and Rose ('36). It ems probable that the discovery of threonine was only through ose's fortitude in the preparation and use of a diet in which the roteins were replaced entirely by mixtures of highly purified amino ids. This is indicated because the chemical properties of threonine e so similar to those of the mono-amino monocarboxy acids, par-cularly the leucines, that it probably would have escaped detection the ordinary procedures for investigation of amino acids in nu-ition.

Attention should be called to the fact that the work on this amino id apparently makes it possible for the first time to rear animals on ets containing mixtures of highly purified amino acids instead of roteins.

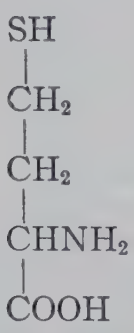
Apparently the only evidence regarding the nutritional role of reonine is the fact that its absence from the diet causes a marked ilure of growth.

Nutritional Relationships between Sulfur-containing A Acids.—It is generally accepted now that methionine is defi indispensable and that in its absence animals die even thoug abundance of cystine is furnished. Nevertheless, under conditio low methionine intake, the addition of cystine to the diet gr improves growth (Rose, '37, l.c.). Thus, it is possible that methio is essential for certain vital functions which cystine cannot s and that cystine can spare methionine for certain functions. Fo ample, in animals which normally produce large amounts of w hair, or feathers, it would seem that dietary cystine in abund might be of considerable advantage since these structures co large amounts of cystine and that it would obviate the necessit synthesizing the cystine from methionine or such other substanc might be convertible into cystine.

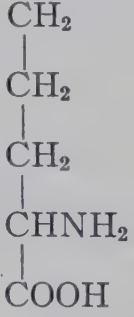
In the light of recent researches, the following compounds rel to cystine and methionine respectively, are attracting attention:



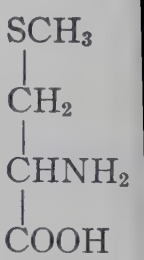
Cysteine



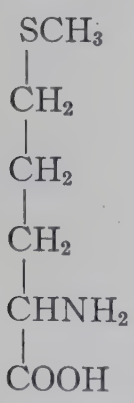
Homocysteine



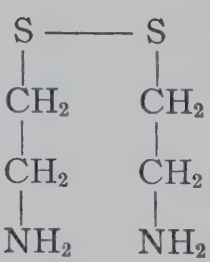
Pentocysteine



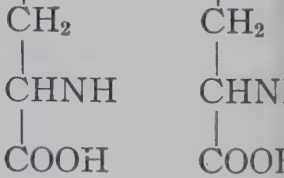
Methyl cyste



Homomethionine



Cystamine



Homocystine

Of these only cystine and methionine have been isolated from protei Dyer and DuVigneaud ('35) found that homocystine could suppo growth on a cystine-deficient diet. But pentocystine and homometh

nine could not be utilized for growth in lieu of cystine, thus demon-rating that the availability of homocystine and methionine for this urpose is not a general property of either disulfide or methylthiol mino acids. However, they were able to show that di-N-methyl-omocystine and N-methylmethionine are able to support growth on cystine-deficient diet. Cystamine appears to be without growth-romoting properties (Jackson and Block, '36). Glutathione, a tripep-de of cystine, glutamic acid, and glycine, is able to replace cystine the diet.

It seems evident, as indicated previously, that the sulfur-containing mino acid of preeminence is methionine (Jackson and Block, '32). pparently, that compound alone can satisfy the entire requirement r sulfur-containing amino acids. The dietary value of other such mpounds seems to depend upon the capacity of the body to con-rt them to methionine, or cystine for certain purposes.

The ability of homocystine to support growth probably is due to s transformation to methionine by the body (White and Beach, '37). ompounds which are convertible into cystine, under conditions of w-cystine intake, may be regarded as of dietary value if the methio-ne intake is not sufficient to furnish adequate amounts of cystine. he nutritional role of sulfur-containing amino acids is a fertile field r investigation.

The effect of various compounds upon the utilization of cystine d methionine is also of interest. Certain substances conjugate with ese amino acids, thus rendering them unavailable to the body and using them to be excreted. Cholic acid and bromobenzene are rticularly effective in this respect (White, '36). Recently, White 37) has shown that iodoacetic acid, when fed to rats restricted to low protein diet, show symptoms of sulfhydryl compound defi-ency, as indicated by the resumption of growth by the addition of ystine or dl-methionine to the diet already containing iodoacetic id.

Sulfur-containing Amino Acids and the Growth of Epidermal ssues.—That large amounts of utilizable sulfur-containing amino ids are necessary for certain animals is indicated by the fact that e percentage of sulfur in clean, dry goose feathers and of sheep's ool is respectively 3.2 and 4.5. The problem of diet in the produc-n of wool is indeed urgent since the quality and amount of wool owth are quite dependent upon the food of the sheep. Bosman 34-35) states that, provided the feed is kept constant, variations in matic conditions produce no effect on the dry weight of wool-ttings or in fibre fineness. He claims, however, that underfeeding duced the scoured fleece by 31.8 per cent, and reduced fibre diam-r by 36 per cent. Subsequent good feeding restored the original

fibre diameter. The necessity of adequate sulfur-containing amin in the diet is further indicated by Marston's ('35) work. H mated the growth of wool on merino ewes, on shaved area compared the growth when the ewes were fed a diet of oat and lucerne hay, with that secured when cystine was added basal ration. The provision of 1 gm. of cystine daily per animal a 14 per cent increase in the growth of wool. It is stated th feeding of methionine gave doubtful results. This is somewha zling since it appears, on the basis of observations of growin that methionine is capable of meeting the entire need for s containing amino acids. It is possible that species differences account for these apparent discrepancies. Certainly it suggests further studies must be made of nutritional relationships be cystine and methionine.

Since wool contains about 13 per cent of cystine, whereas proteins contain less than 1 to 3 per cent, Marston and Robe ('28) advanced the important hypothesis ". . . that the carrying c ity of any country for sheep may very probably be determine the capacity of its pasture plants to produce cystine."

If wool-producing animals are dependent, in any degree, methionine as a source of wool sulfur, present analytical data not indicate that methionine deficiency in the diet might be a l ing factor in wool production. Barritt ('34) found that the m onine content of wool amounts to 0.44 to 0.66 per cent of its weight and methionine sulfur represents only 2.4 to 4.8 per cen the total sulfur content. Methionine deficiency does not seem li to occur in animals or in man if the diet is composed of nat foodstuffs.

Several studies have been made on the relationships between die cystine and the growth of hair in rats. Rats of Smuts, Mitchell Hamilton ('32) on a cystine-deficient diet had a subnormal amo of cystine in the hair and the medullated fibres were abnor Also, hair growth was definitely inhibited. A diet deficient in ly inhibited body growth and hair growth, but did not reduce the tent of cystine in the hair.

Of interest here is the study of Martin and Gardner ('35) the effect of sulfhydryl compounds on the growth of hair in ge cally hairless rats. Five milligrams of cystine administered dail such rats stimulated the growth of a hairy coat within one mo but it was lost during the next two weeks. Higher doses faile initiate a regrowth of hair. However, hair growth stimulated cysteine continued as long as the experiment lasted, which was weeks. Glutathione had no stimulative action. It is unfortunate these experiments were not continued longer. since the effects

lfur-containing amino acids, under such conditions, are of consid-
able significance regarding the genetic mechanism of hypotrichosis.
owever, these brief observations do suggest that hereditary hypo-
chosis might be regarded as an "inborn error of metabolism" as
the case of pentosuria, cystinuria, and alkaptonuria.

The possible relationship of dietary deficiencies of sulfur-containing
nino acids to loss of hair in humans is indeed problematic. As
phasized by Brown and Klauder ('33), loss of hair is not neces-
ily associated with low sulfur in the diet. At this time there is
t sufficient information to warrant unequivocal conclusions regard-
g sulfur-containing amino acids and hair growth in humans.

Sulfur-containing Amino Acids and Tissue Growth.—It has been
stulated by Hammett ('33) that the natural regulation of growth
increase in cell number is brought about by the essential chemical
uilibrium between the sulfhydryl group, –SH, and its partially
idized derivatives. It is claimed that the sulfhydryl group accelerates
owth and the partially oxidized derivatives of such compounds re-
d it. Hammett has employed as sources of sulfhydryl, reduced
utathione, parathiocresol, thiophenol, and thio-glucose.

Voegtlin, Johnson and Thompson ('36) have confirmed earlier
servations showing that deficiency of cystine or methionine, or
h, inhibit the growth of mice. The growth of neoplasm, i. e., mam-
ary carcinoma, is retarded in mice restricted to a diet deficient in
tine and methionine. However, as in other cases of tumor inhi-
ion by dietary deficiencies, the growth of the mice also is inhibited.
e addition of cystine or glutathione to the deficient diet causes
stimulation of tumor growth, concomitant with resumption of
owth by the host. In no case has it been demonstrated that tumor
owth can be retarded or controlled by dietary measures without
using simultaneous retardation of normal tissue growth and func-
n. This does not imply, however, that diet is without significance
tumor growth and tumor susceptibility.

Sulfur-containing Amino Acids in Processes of Detoxication.—
steine and glutathione play a prominent role in the bodily proc-
es of detoxication. The former compound, although not a signifi-
nt constituent of the normal diet, may be easily produced in the
dy by reduction of cystine. Detoxication may possibly occur by
ans of several different reactions involving cystine, glycine, and
tamic acid, all of which are components of glutathione.

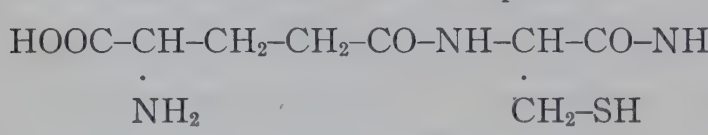
Sullivan and Hess ('34) are of the opinion that sulfur-containing
ino acids are especially important in arthritis and perhaps other
cases of a degenerative nature. A basis for this opinion is the low
tine content of finger nails. They found low values in 65 per cent
patients with arthritis. The normal range is reported to be 10.2

to 13 mg. per cent; the average being 11.8 mg. In patien arthritis the values were 7.2 to 13.1 mg. per cent, with an of 9.8 mg. per cent. Since the amounts of arginine, histidi lysine are normal in these cases, the low values for cystine ar preted as an indication of the existence of an inadequate mec for detoxication, suggesting, therefore, a significant relations the arthritic state of the patients. Further support of this view report that sulfur therapy is followed by an increase in the of nail cystine.

In view of the present evidence it appears that deficiency of containing amino acids, due perhaps to inadequacy of the diet, in absorption, or increased metabolic requirement, is a contri factor in degenerative diseases such as arthritis. However, the p evidence is merely presumptive and should be regarded as such further proof is obtained.

Glutathione.—The tripeptide, glutathione, was isolated by kins in 1921 and its structure was established by Harington and in 1935 by synthesis. However, previous investigations of the is substance had indicated its composition and structure. Several ers contributed to the latter discovery.

The structure of the reduced compound is:



Glutathione may exist in the body as the reduced (sulfhydry oxidized (disulfide) compound. Its participation in enzymatic esses is definitely proved but the specific roles of this reve sulfhydryl-disulfide substance are not known. In all probabilit tripeptide is synthesized in the body from its constituent amino This is especially indicated by the work of Rose whose basal for the study of amino acids is entirely devoid of glutathione e for the small amount contained in the vitamin B concentrate.

Specific Functions of the "Indispensable" Amino Acids.—O the fields for future research in nutrition is the detailed inve tion of the effects of specific deficiency of each of the indispen amino acids. At present there is relatively little definite know to record. The question is still an open one whether starvatio a simple amino acid produces a unique syndrome.

Some indication of what might be observed in specific amino deficiencies is revealed in Weichselbaum's ('35) study of cystine methionine deficiency in the rat. It was observed that rats on a man-Merrill (1925) cystine-methionine deficient diet ". . . bega sicken and die during the sixth week. They refused food and be

ggish; within the next two or three days they exhibited a marked vature of the spine, showed clear signs of icterus, cyanosis of the t, ears, and nose, became cold and had a partial paralysis of the oat which prevented swallowing. Death occurred usually within ee days of the first obvious signs. Postmortem examination showed signs of pneumonia but definite haemorrhages throughout the er."

Also, the important observations of valine deficiency by Rose sug- ts further the need of investigations of specific amino acid defi- ncies. As pointed out by Rose ('37, l.c.), "Its (valine) absence m the diet induces a profound failure in growth accompanied by development of symptoms which are unlike any we have en- ntered in other types of deficiencies. Animals deprived of valine come sensitive to touch and manifest a severe lack of coordination movement." The study of specific amino acid deficiency will be- me interesting and profitable indeed as soon as it becomes possible prepare basal diets containing smaller amounts of interfering amino ds and to secure pure amino acids at lower cost.

REFERENCES

ULDER (1839). J. prakt. Chem., *16*, 129.
NITZ and NORTHROP (1933). Science, *78*, 558.
HEORELL (1934). Biochem. Ztschr., *272*, 155.
LIN (1905). Am. J. Physiol., *13*, 117.
CKERY and SCHMIDT (1931). Chem. Rev., *9*, 169.
EDBERG (1934). Science, *79*, 327.
YCKOFF (1936). Science, *84*, 291.
ARD and WYCKOFF (1937). Science, *85*, 201.
ANLEY and WYCKOFF (1937). Science, *85*, 181, 390.
RINCH (1937). Science, *85*, 566; Nature, *139*, 972.
OWFOOT (1935). Nature, *135*, 591.
RGMANN and NIEMANN (1937). Science, *86*, 187.
ODGETT (1935). J. Am. Chem. Soc., *57*, 1007.
NGMUIR, SCHAEFER and WRINCH (1937). Science, *85*, 76.
OCK and VICKERY (1931). J. Biol. Chem., *93*, 113.
OCK (1933a). J. Biol. Chem., *104*, 339.
OCK (1933b). J. Biol. Chem., *103*, 261.
OCK (1934a). J. Biol. Chem., *105*, 455.
OCK, DARROW and CARY (1933). J. Biol. Chem., *104*, 347.
CKERY and WHITE (1933). Proc. Soc. Exper. Biol. & Med., *31*, 6.
OCK (1934b). J. Biol. Chem., *105*, 663.
STENEYS and BORSOOK (1930). Physiol. Rev., *10*, 110.
NENE and VAN SLYKE (1908). Biochem. Ztschr., *13*, 458; *16*, 202.
ARD (1926). J. Biol. Chem., *71*, 477.
COCK (1936). Physiol. Rev., *16*, 1.

Henriques and Gjaldbäk (1911). Ztschr. f. physiol. Chem., 7
(1912). *81,* 439.
Carey and Meigs (1928). J. Biol. Chem., *78,* 399.
Berg and Rose (1929). J. Biol. Chem., *82,* 479.
DuVigneaud, Sealock and Van Etten (1936). J. Biol. Chem., *112,*
Schenck and Wollschnitt (1933). Arch. f. exper. Path. u. Phar
170, 151.
Lee and Lewis (1934). J. Biol. Chem., *107,* 649.
Calvery and Titus (1934). J. Biol. Chem., *105,* 683.
McFarlane, Fulmer and Jukes (1930). Biochem. J., *24,* 1611.
Patton and Palmer (1936). J. Nutr., *11,* 129.
Csonka (1937). J. Biol. Chem., *118,* 147.
Harrow and Sherwin (1935). "A Textbook of Biochemistry."
Saunders Co., Philadelphia.
Rose (1938). Physiol. Rev., *18,* 109.
Griffith (1934). J. Biol. Chem., *105,* xxxiii.
McCoy and Rose (1937). J. Biol. Chem., *117,* 581.
Abderhalden (1922). Arch. f. d. ges. Physiol., *195,* 199.
Rose (1937). Science, *86,* 298.
Womack and Rose (1936). J. Biol. Chem., *116,* 381.
Bunney and Rose (1928). J. Biol. Chem., *76,* 521.
Hopkins (1916). J. Chem. Soc. (British), *109,* 629.
Windus, Catherwood and Rose (1931). J. Biol. Chem., *94,* 173.
Rose and Cox (1924). J. Biol. Chem., *61,* 747.
Stewart (1925). Biochem. J., *19,* 1101.
Arnold, Kline, Elvehjem and Hart (1936). J. Biol. Chem., *116,* 6
Klose, Stokstad and Almquist (1938). J. Biol. Chem., *123,* 691.
Scull and Rose (1930). J. Biol. Chem., *89,* 109.
McGinty, Lewis and Marvel (1924). J. Biol. Chem., *62,* 75.
Berg and Dalton (1934). Proc. Soc., Exper. Biol. & Med., *31,* 709
Berg (1936). J. Nutr., *12,* 671.
Ackroyd and Hopkins (1916). Biochem. J., *10,* 551.
Cox and Rose (1926). J. Biol. Chem., *68,* 781.
Fishman and White (1936). J. Biol. Chem., *113,* 175.
DuVigneaud, Sifferd and Irving (1937). J. Biol. Chem., *117,* 589.
Osborne and Mendel (1912). J. Biol. Chem., *12,* 81.
Abderhalden (1913). Ztschr. f. physiol. Chem., *83,* 444.
Jackson (1927). J. Biol. Chem., *73,* 523.
Jackson (1929). J. Biol. Chem., *84,* 1.
Berg, Rose and Marvel (1929). J. Biol. Chem., *85,* 207.
DuVigneaud, Sealock and Van Etten (1932). J. Biol. Chem., *98,* 5
Gordon and Jackson (1935). J. Biol. Chem., *110,* 151.
Abderhalden (1915). Ztschr. f. physiol. Chem., *96,* 1.
Totani (1916). Biochem. J., *10,* 382.
Embden and Baldes (1913). Biochem. Ztschr., *55,* 301.
Shambaugh, Lewis and Tourtellotte (1931). J. Biol. Chem., *92,* 4
St. Julian and Rose (1932). J. Biol. Chem., *98,* 457.
Sure (1924). J. Biol. Chem., *59,* 577.

E (1932). Yale J. Biol. Med., *4*, 519.
COY, MEYER and ROSE (1935). J. Biol. Chem., *112*, 283.
YER and ROSE (1936). J. Biol. Chem., *115*, 721.
ER and DUVIGNEAUD (1935). J. Biol. Chem., *109*, 477.
KSON and BLOCK (1936). J. Biol. Chem., *113*, 135.
KSON and BLOCK (1932). J. Biol. Chem., *98*, 465; (1931). Science, *74*, 14.
ITE and BEACH (1937). J. Biol. Chem., *122*, 219.
ITE (1936). J. Biol. Chem., *112*, 503.
ITE (1937). Science, *86*, 588.
MAN (1934-35). Nutr. Abstr. & Rev., *5*, 825.
RSTON (1935). J. Agric. Soc. (Australia), *25*, 103, 113.
RSTON and ROBERTSON (1928). Australia, Inst. Sci. Ind. Bull. 39.
RITT (1934). Biochem. J., *28*, 1.
TS, MITCHELL and HAMILTON (1932). J. Biol. Chem., *95*, 283.
RTIN and GARDNER (1935). J. Biol. Chem., *111*, 193.
WN and KLAUDER (1933). Arch. Dermatol. & Syph., *27*, 584.
MMETT (1933). Science, *77*, 190.
EGTLIN, JOHNSON and THOMPSON (1936). U. S. Pub. Health Reports, *1*, 1689.
LIVAN and HESS (1934). J. Bone & Joint Surg., *16*, 185.
ICHSELBAUM (1935). Quart. J. Exper. Physiol., *25*, 363.

CHAPTER

VI

Nutritional Value of Proteins and the Effe of Variations in the Level of Dietary Prote

THAT THE NUTRITIONAL value of proteins varies g depending upon their amino acid composition and physical con was not clearly realized until it was emphasized by Rubner in Shortly thereafter the work of several investigators, particularly cock and Hopkins in England, and Osborne and Mendel in Am demonstrated, for instance, that while rats could not live on in which zein, the chief protein of maize, was the principal s of protein, they could do so if tryptophane were added. It was shown that several purified proteins could be greatly enhance respects their nutritive value, when they were supplemented certain amino acids. Furthermore, it was learned that combina of certain proteins in the diet produced better effects than the proteins when fed individually and in similar concentrations. I therefore become of practical as well as academic importance to mate the nutritional value of purified proteins and protein-conta foods. This has necessitated an understanding of the various r for protein in the body, particularly in relation to growth, m tenance, reproduction, and lactation.

For these reasons it is necessary to consider the physiological s of the organism, whose protein requirements are being consid if the nutritional value of protein used in promoting that stat to be reliably assessed. This is of some importance since, for exa it has not been satisfactorily determined whether the amino requirements for maintenance of an animal are identical with different from those for growth. With respect to maintenance must regard protein nutrition as involving both the provision the utilization of such amino acids as cannot arise in the through synthesis, and are needed for the replacement of t

roteins which undergo degradation in the chemical processes of life. But in addition to these needs, certain amino acids are apparntly required for the special purpose of serving as precursors from hich hormones are synthesized. Thyroxine and epinephrine are lmost certainly derived from tyrosine or phenylalanine. It is not nown what happens to the proteins resulting from the endogenous egradation of tissue nitrogenous compounds. Clarifying information needed as to whether molecules are completely degraded by hydrolsis, or whether some other type of chemical change occurs. It is nown that when an animal is restricted to a diet which provides ufficient energy in the form of carbohydrates and fats, and all the ssential mineral elements and vitamins, it continues to excrete hourly nd daily a certain irreducible quota of nitrogenous end-products, two epresentative ones being creatinine and neutral sulfur compounds. ut regardless of the uses made of protein in maintenance and other nctions, in a practical sense, it is only necessary to ascertain the egree to which given proteins function in meeting those needs condered as a whole.

The Nutritional (Biological) Value of Proteins.—In order to etermine the nutritional value of a particular source of protein, either isolated protein or a naturally-occurring mixture, it is logical to ppose that it is only necessary to determine the minimum amount such protein which will just serve to meet the endogenous need, d keep the animal in nitrogen equilibrium. This food protein would place any protein degraded in the tissues, that used to form epirmal structures and that secreted in the digestive fluids as mucus, hich, in part at least, escapes reutilization and is lost in the feces. McCollum and Steenbock ('12) studied the utilization of zein and gelatin by young swine which were fed starch, a salt mixture, d water. In one pig, for example, the nitrogen excreted in the ine was about 2.6 gm. daily, after the value had become reduced an almost constant daily output. When the pig was fed this nount of nitrogen daily, in the form of zein, the urinary nitrogen tput during eight successive days was 2.48, 2.75, 2.98, 2.62, 2.56, 7, 3.11, and 3.85 gm. respectively. In a succeeding period of 5 days animal was fed only the basal ration of starch, salts, and water. this period the output of urinary nitrogen was 3.07, 2.37, 3.69, 3, and 2.94 gm. per day. Since the fecal nitrogen output remained nost constant during these periods it appears that the animal utilized considerable part of the nitrogen of this "incomplete" protein for pair or maintenance purposes. These studies were made when little, anything, was known about vitamins and some other dietary fac-s. Hence the diet was inadequate with respect to several factors. verthcless, it seems very doubtful whether this fact invalidates the

conclusion that incomplete proteins can serve, to some degr tissue sparers when given to an animal as a sole source of acids. It is not possible to decide whether the effect of feedi incomplete protein under these conditions is due to the mass effect of keeping up the amino acid concentration of the bloo tissues, even with certain ones lacking which cannot be synth But on the basis of this consideration, and others which mig given, it is obvious that the nutritional value of a protein, o tein mixture, for growth cannot be estimated by feeding at th dogenous plane of protein metabolism.

Without further consideration here of protein metabolism, is fundamental in regard to the estimation of the nutritional of proteins, it suffices to state that two general procedures ca used. They are usually referred to as the nitrogen balance me and the growth method. Each of these will be considered i much detail as space will permit. However, it is important to re that a protein, or mixture of proteins, might not satisfy the req ments for lactation, as an example, in the same degree that it sat the needs for growth. There is no definite evidence in oppositio this premise, but theoretically it is valid since, for example, the ar acid mixture required by the mammary gland to synthesize might not be similar to that required in the young animal to pro growth.

There are no very satisfactory procedures for determining nutritional value of proteins except for growth and even in t there is no general agreement. The factors involved have been tensively discussed. Mitchell and Hamilton ('29) have canvassed entire subject and Boas Fixsen ('34-35) has succinctly reviewed methods applicable to this problem.

Nitrogen Balance Methods for Determining the Nutritive Va of Protein.—In 1909 Thomas devised a procedure wherewith the va of a protein could be estimated from a short-term nitrogen bala experiment, using rats or other suitable experimental animals. vious faults in the method were soon recognized. In 1924 Mitc ('24) published a critical study of the nitrogen balance technic proposed modifications which were claimed to constitute quate improvements. Since then Chick and Roscoe ('30), Boas sen and Jackson ('32), and others, have described further modif tions.

Space will not permit a thorough discussion of the method, it is important that the student have presented at least a brief line of it. As described by Mitchell ('26), "The experiments on involve direct determinations of the amounts of nitrogen in the f and in the urine, and indirect determinations of the fractions of

l nitrogen and of the urinary nitrogen that are of dietary origin. biological value of the protein is taken as the percentage of orbed nitrogen (nitrogen intake minus fecal nitrogen of dietary in) that is not eliminated in the urine."

As stated further by Mitchell, "Obviously the uncertainty of the hod is related to the factoring of the fecal and urinary nitrogen food and body components. Since no direct method is available, rect methods must be relied upon. There is reason to believe that n estimations may be based on the values for the daily excretion urinary and fecal nitrogen on adequate amounts of a nitrogen- ration. The 'metabolic nitrogen' of the feces on a protein-contain- diet . . . is related to the amount of food consumed and may be sured by the total excretion of fecal nitrogen on a nitrogen-free . The latter may be used with the most confidence when the ghage' content of the nitrogen-free diet approximates that of the ein-containing diet. The 'endogenous nitrogen' of the tissues is ted to the body weight of the animal, and may be measured by total urinary nitrogen on an otherwise adequate nitrogen-free . Both of these values are, of course, to be determined only after eriod of preliminary feeding of sufficient length to insure equi- ium between the animal and its food.

'The method may be illustrated by the following analysis of actual metabolism study on a rat receiving a ration containing roximately 4 per cent of a mixture of proteins derived from corn tankage. The rat consumed an average of 56.9 mgm. of nitrogen day in a 7-day collection period. On this diet, the daily excre- of fecal nitrogen was 27.6 mgm., but of this nitrogen it is nated from the fecal nitrogen excreted on a nitrogen-free diet, 21.7 mgm. were derived from the body, leaving only 5.9 mgm. ained in the indigestible food protein. Hence, the rat was receiv- daily $56.9 - 5.9 = 51.0$ mgm. of absorbed nitrogen. During this od, the excretion of urinary nitrogen was 48.6 mgm. daily, of ch 37.7 mgm. were the result of the catabolism of the body's tissues (estimated from the excretion of urinary nitrogen on a ogen-free diet). Hence, only $48.6 - 37.7 = 10.9$ mgm. of the ab- ed dietary nitrogen were wasted in metabolism, and $51.0 - 10.9$ 0.1 mgm. were retained in the body. The biological value of the ein, therefore, is equal to $100 \times (40.1 \div 51.0) = 79$."

Mitchell's formula which expresses the nutritional (biological) value protein is as follows:

$$100 \times \frac{\text{Body Nitrogen Spared}}{\text{Food Nitrogen Absorbed}}$$

It should be stated further that the basal diet used in this r contains sufficient carbohydrate and fat to satisfy the energy r ments; thus tending to prevent the catabolism of body prot energy. The diet must be complete as respects mineral elemen vitamins, these being supplied as the pure substances or the potent concentrates obtainable, so that the only inadequacy w with respect to protein. However, as indicated in Tables I, I III (see appendix), on the nutritive value of proteins, it w observed that practically all determinations made by the ni balance method, as well as others, have not satisfied this criteri most cases inadequate amounts of vitamin B-complex were pr or else the amount of nitrogen introduced with the vitamin s ment was sufficiently large as to probably supplement or augme test protein.

Nevertheless, this method does furnish useful information re ing the nutritive value of proteins. It should be recognized, hov that there are some valid and significant objections to its use. O these relates directly to technical problems of determining nit balance under such conditions. Obviously feces and urine mus only be collected quantitatively, but they must be accurately rated. Also, even slight contamination of the excreta with the will vitiate the results. Moreover, the so-called endogenous nitr output must be determined for each experiment since it varies only from animal to animal, but in the same animal at diff times.

In order to ascertain the endogenous nitrogen output chara istic of the test animal it is necessary to keep the animal on a nitro free diet to bring it into a condition where the endogenous prevails. This preliminary period must not be prolonged to a which causes loss of appetite.

The estimation of fecal nitrogen of endogenous origin pre practical difficulties. A part consists of undigested protein, or tein which has been fixed in the intestine as bacterial protein which escapes absorption; and a part consists of unabsorbed pr excreted into the intestine with the digestive juices, which is lo the feces. If the diet contains much indigestible material, whic creases the water content, the latter fraction is increased. Obvi this loss should be kept as low as possible. In general, the endoge fecal nitrogen has been found to be proportional to the food in

Since the nutritional value of proteins, as generally determ by the nitrogen balance method, are expressions of the utilizatio absorbed protein, it should be evident that a protein might b very poor quality, owing to low digestibility, yet rate a relatively

ritional value. This is shown in the data of Seegers and Mattill 5), who compared the nutritional value of dried liver with that heated dried liver, using the nitrogen balance method. With their al diet, containing 7.2 per cent of whole dried liver, young rats ghing 40 gm. grew to an average of 165 gm. in 45 days. The ne diet with heated liver induced rats of similar initial weight increase to an average of 110 gm. in 50 days. In contrast to these ge differences in the growth-promoting value of the two proteins, was found that the nutritional value of whole dried liver, was (average of eight experiments) and that of heated liver was 51 erage of eight experiments). It is apparent that the ability of the mals to utilize the proteins from these two sources for growth is y approximately expressed by the nutritional value of the protein letermined by the nitrogen balance method.

Growth and Weight Maintenance Methods.—In order to deter-ne the value of a protein in the promotion of growth the most ural method of making that determination is the actual measure-nt of growth occurring with a given intake of protein. The prob-s of magnitude, in this procedure, are (a) food consumption, (b) gth of experimental period, and (c) level of test protein in the t. Their significance will appear in the discussion to follow.

The extensive and important studies of Osborne and Mendel were ed entirely on some form of growth performance with a given ake of protein. Osborne, Mendel and Ferry's ('19) method relates growth obtained to the intake of protein, and expresses the nutri-nal value (X) of the protein by the ratio

$$X = \frac{\text{Gain in Weight}}{\text{Intake of Protein}}$$

pointed out by Boas Fixsen ('34-35, l.c.), "Since, before any pro- can be used for growth, the maintenance requirement must be sfied, the value of X will rise as the protein intake is increased il an optimum is reached at which the intake of protein is icient to allow the maximum rate of growth. Thereafter, with fur- increase in protein intake, the value of X declines, the surplus tein being used for energy requirements." Osborne, Mendel and ry attempted to determine the level at which a given protein uld be fed in the diet in order to obtain the maximum value X. Most workers, however, have fed the test protein at an arbi-ily fixed level. This is usually from 5 to 12 per cent. If fed at her levels appreciable and increasing amounts are used to satisfy

energy needs and if fed at lower levels appreciable and i amounts are used to satisfy maintenance needs.

In this method, as in the nitrogen balance procedure, stri tion must be given to the provision of adequate energy, a hydrate and fat; and adequate vitamins and minerals with introduction of appreciable protein or other nitrogenous ma

It seems unnecessary to place a low estimate on the re this type of experimental comparison of proteins. They yield which do compare proteins in an intelligible and practical w we should never be able to deduce these values from the n balance procedures.

McCollum and Shukers (McCollum and Simmonds, '29 modified this general procedure by determining the actual ($N \times 6.25$) retention which is possible when a given protein sumed at an arbitrary level of intake. Three groups of you are selected which are strictly comparable with respect to age, and genetic composition. Group 1 rats are killed, the contents digestive tracts removed by washing, and the entire carcass hashed and analyzed for total nitrogen. The remaining two are fed for 28 and 56 days respectively, on screens to prevent to feces, the food consumption being carefully determined. Th nitrogen contents of their carcasses is determined as in the c group 1 rats. From these data, including the protein consum it is obviously possible to estimate the per cent of ingested p which is used to form tissue protein ($N \times 6.25$). The per ce retained represents the nutritional value of the food protein jected to test.

The advantage of this method over that of growth proce such as those of Osborne and Mendel, is that the actual p (nitrogen) retention is determined. This may be of some impo since it has been shown that the composition of tissues may considerably during growth owing to differences in dietary in ents. With reference to the nitrogen balance methods it also has advantages. The animals are under less stress as obtains in c tions imposed by the quantitative collection of excreta. Als procedure requires less technical work. It expresses the nutri value of protein in practical terms, i. e., the percentage utiliz of ingested protein for growth rather than the percentage u tion of absorbed nitrogen for the sparing of body nitrogen nitrogen starvation.

The following table provides a comparison of values obt by the Osborne and Mendel growth method, the McCollum Shukers nitrogen retention method, and the Mitchell nitrogen ba method:

COMPARISON OF THE NUTRITIONAL VALUE OF PROTEINS AS DETERMINED BY THREE DIFFERENT METHODS

SOURCE OF PROTEIN	PER CENT PROTEIN IN DIET (N × 6.25)	AVERAGE NUTRITIONAL VALUES		
		Osborne and Mendel	McCollum and Shukers	Mitchell
ole wheat	8.25	1.18	25.0	82
nhaden meal	8.7	1.25	21.1	72.5
nhaden meal	12.1	1.11	22.0	67.3
nhaden meal	14.4	0.98	20.9	60.5
nhaden meal	17.8	1.30	24.7	68.6
ole wheat 67.5, ... sein 10.0, and whole milk owder 10.0	17.4	1.67	34.3	71.9
ole wheat 35.5, ... sein 5.0, and whole milk owder 10.0	10.4	1.86	37.6	85.8

Paired Feeding Method.—This method, devised by Mitchell and dles ('29-30), is generally regarded as a particularly suitable pro- ure in studies of the supplemental relationships between proteins the amino acids in which they might be deficient. Of course it other applications. At the beginning of an experiment young are paired with respect to weight and genetic capacity. If, for mple, the problem is the determination of cystine deficiency in rotein one rat is given the basal diet containing the test protein the food intake is carefully determined. The mate to this rat iven the same basal diet and test protein plus cystine, but the d intake is restricted to the amount ingested by the first rat. If, r a given period of time, the rat receiving added cystine weighs e than its mate it is concluded that the protein in question is cient in cystine or that the cystine is unavailable in adequate unts. The degree of deficiency is determined by the differences veight of the two rats. Thus, numerical results are obtained and erences in palatability of test proteins are controlled. In order to e the data statistically valid eight or more pairs of rats are used ach assay.

Boas Fixsen ('34-35, l.c.) has called attention to the limitations he paired feeding method for comparison of the utilization of eins. The principal question is whether an animal which is not wed to satisfy its appetite is strictly comparable, for the purpose ie paired-feeding method, with one which eats all it wants.

McCollum Long-term Studies of Protein Foods.—McCollum and ssociates ('21) attempted to compare the relative values of pro-

teins from different sources by feeding a series of groups on comparable basal diets containing equal proportions of d proteins. The results were judged on the basis of growth, f lactation, infant mortality, and longevity. Boas Fixsen ('34-3 has criticized these studies. She states: "Where so many fact involved, the assessment of results such as these must be la matter of individual judgment, some proteins giving better for one function, others for another. It seems unjustifiable, a assume that all the dietary essentials are yet recognized, parti those whose absence would be felt only after long periods of vation. Since no records were made of food intake, it is imp to ascertain whether the intake of the rats receiving the differen was comparable."

Her criticism seems to justify the too common belief that is unique value in knowing the nutritional values of the pr of different foods apart from their palatability, and hence the sumption of food by animals fed different natural products also that the point of singular interest is the determination o nutritional values of proteins for growth. It is, of course, a m of individual interest whether it is desirable to know the subse physiological history of animals fed a comparable basal diet supplying proteins from different sources, as shown by their gr fertility, capacity to secrete milk, and longevity. The experimen the type we are considering do show the comparative values o ferent foods, expressed in terms of their protein, as respects effects on the life histories of the animals. They do not enable to express the nutritional values of these proteins numerically, were never claimed to have this merit.

Biological Availability of Protein in Foodstuffs.—Before ing protein can be absorbed and utilized by the body it must be h lyzed (digested) into its constituent amino acids, or possibly si peptides. In the case of certain foodstuffs the protein is compl hydrolyzed, under physiologic conditions, but others are only tially reduced to the amino acid stage. The protein of eggs is a example of the former and those of most legumes represent one of the latter. This, however, does not constitute the only crit of nutritional quality, as subsequent discussion in this chapter reveal.

Soy beans are useful examples of protein apparently containin of the amino acids essential for good health. Yet, upon biolo analysis, they have consistently shown low nutritive values. Bu demonstrated by Hayward, Steenbock and Bohstedt ('36a), the application of heat, sufficient to produce a temperature of 140 to C. for 2½ minutes, practically doubles the nutritive value. A

r improvement in nutritive value was demonstrated by supple-
ntation of this protein with 0.3% l-cystine. This suggests that
tine or its equivalent may exist in the raw soy bean in a form
ich is not available to the animal and that the appropriate appli-
ion of heat is sufficient to render the cystine available. Apparently
logous situations exist with respect to several other protein foods,
ticularly in meats which contain relatively large amounts of con-
tive tissue.

Another factor is the effect of roughage on protein utilization
unnell, et al., '36). Numerous studies of this subject have been
de, but opinion seems to differ concerning the significance that
uld be attached to it. Adolph and Wu ('34), for example, claim
. a slight tendency toward a lowered degree of protein digesti-
ty only when the fiber was ingested in an abnormally large
ount." They suggest that lowered values for protein digestibility
a given diet result only when the food material passes through the
nentary tract with abnormal rapidity. In view of the present evi-
ce this appears to be a reasonable conclusion.

A third factor which is probably of greatest practical importance
hat of heat, particularly dry heat. This will be considered subse-
ntly along with data on the effects of ultraviolet irradiation and
mination.

Space here is too limited to permit much consideration of the
ct upon digestion of carbohydrate and protein mixtures. But brief
nment seems necessary since there is a surprisingly large class of
people who seem to be of the opinion that mixtures of protein
carbohydrate interfere with gastric digestion. A large amount
evidence may be cited in refutation of this faddist's belief. Two
dies of particular value are those of Rehfuss ('34) and those of
y, Gershon-Cohen and Fels ('36), who found no evidence what-
ver of incompatability between carbohydrate and protein digestion.

Nutritional Value of Common Proteins.—It has been emphasized
t the nutritive value of protein is ultimately dependent upon the
no acid content. Moreover, consideration has been given to the
tionship between nutritive value and such factors as protein diges-
lity and the level of protein in the dietary. These variables are
sidered in estimating the nutritive value of proteins.

In practical dietetics neither animals nor man are ever restricted
a single protein as a source of the necessary amino acids. Hence,
a upon the nutritive value of individual proteins are actually of
utilitarian value than those of the supplementary relationships
veen such dietary essentials. But it is advantageous to consider
, as a basis for subsequent attention, the data on individual pro-
s. These are summarized in tables I and II (Appendix). How-

ever, one should be particularly critical in the use of this a
sequent tables of proteins in nutrition. One must realize tha
is no general agreement concerning methods of estimating th
tive value of proteins. Consequently it has been necessary to
an arbitrary policy in preparing such tables. At best the nu
data constitute only approximations of the actual nutritiona
of the proteins which they represent. Certainly one is not
make deductions from them concerning the relative value
teins in such general functions as growth, maintenance, reprod
and lactation. It must be assumed, until more refined analyti
tinctions can be made, that the value of a protein in any
these functions is approximately similar with respect to all of

In general, as revealed by table II (Appendix), proteins of
origin have a higher nutritive value than those of any other
This is understandable, particularly in the case of milk and
since these proteins must provide all the amino acids requir
the young mammal or developing chick.

Whole eggs, which constitute a mixture of proteins, have a h
nutritive value than any other source of protein known. Hov
egg white, separated from the yolk, gives a rather low valu
shown by the table.

Considerable difference of opinion has existed concerning the
of casein and lactalbumin, the principal proteins of milk. It ap
that lactalbumin constitutes a better source of essential amino
than casein alone. Undoubtedly the method of isolating these
teins influences in considerable measure their nutritive value.
probably accounts for some of the early conclusions, 10 to 15
ago, that lactalbumin is an inferior protein. Dry heat is know
lower the nutritive value of proteins if it is applied even for
intervals of time. It appears that the limiting factor in casein is cy
(Mitchell, '23-24a), although that amino acid is not essential
adequate amount of methionine is present. Hence it is a com
practice to fortify casein with 0.05 to 0.10 per cent of cystine in st
of rats if it is desired to feed this protein at a low level.

It is generally found that the proteins of liver, kidney, and
have higher nutritive values than those of skeletal muscle meats
pointed out by Mitchell, Beadles and Kruger ('27), the nutrit
value of meat is inversely dependent, in considerable measure,
its content of connective tissue. Hence some of the cheaper cu
meat, which contain large amounts of connective tissue, are disti
less valuable as sources of proteins. However, there is some su
mentary relation between connective tissue and the protein of mu
It appears that the nutritive values of sweetbreads, tripe, beef c
meat, and ox lips are definitely lower than those of skeletal mus

oagland and Snider ('36) claim that there appears to be scarcely y difference between veal, ox, and hog brains, ox and hog tongues, spleens, dried milk, and skeletal muscles of ox, hog, and sheep. itchell claims, however, that he consistently obtains a higher nutri- e value for milk proteins than for meat proteins.

A consideration of proteins derived from animal tissues would t be adequate unless some attention were given to gelatin. This otein, derived from the collagen present in white fibrous tissue, s been the subject of controversy, regarding its nutritional sig- ficance, since the early part of the nineteenth century. It is indeed toriously deficient in certain indispensable amino acids and, con- quently, will not even support life when it constitutes the only urce of amino acids in the diet. However, it appears to be well lerated and may be of value as an appetizer. But, as concluded by e Council on Foods of The American Medical Association ('36), it not established that gelatin aids in the digestion of milk or the eatment of myopathies. For the latter purpose gelatin has been in me favor owing to its high content of glycine, the amino acid alleged be of benefit in myopathies.

On the basis of dietary studies made several years ago by the . S. Department of Agriculture it appears that white flour (wheat) ntributes almost 30 per cent of the protein in the average American etary. There is no reason to assume that the value has changed aterially since that time. Owing to the prominent place of wheat, t, and corn products in the dietary of both man and agricultural imals these and certain other cereals should be given some atten- on here.

As is indicated by the tables, all cereal proteins have lower nutri- onal values than those, in general, of meat and animal products. or example, according to Mitchell and Carman's data ('26), the utritional values of white flour, whole egg, egg albumin, milk, veal, d beef are respectively, 52, 94, 83, 85, 62, and 69. Boas Fixsen et al. 34) claim that there is scarcely any difference between the nutritive lues of whole wheat and whole corn but Mitchell's ('23-24b) data dicate slight superiority of oats over corn. Mattill ('30), using the trogen balance method, obtained results indicating approximately e same differences between wheat and oats. The values for wheat dosperm and whole wheat were essentially alike, but those of pre- oked oats were definitely higher. In the interpretation of these ta, obtained by means of the nitrogen balance procedure, it should remembered that what was determined was the percentage of sorbed protein used in anabolism. The amount wasted through ilure of digestion or absorption was ignored in expressing the umerical ratings.

The legumes, in general, contain rather high percentages o tein and in some parts of the world they are the chief sou dietary proteins. In this country, soy bean protein is of consid interest as an animal food, but, as indicated previously, the nu value is relatively low unless it is cooked or supplemented cystine, procedures which are obviously impractical. Data, su are available on peas, peanuts, and other legumes may be fou table I.

Tubers contain significant amounts of protein but neither nor sweet potatoes are particularly valuable as sources of all the tial amino acids.

Yeasts, molds, and other microorganisms probably could be upon to furnish protein, as well as other dietary essentials, if pr sources should become limited. It has been demonstrated that can grow on diets containing yeast as the sole source of pro Skinner ('34) believes that all the essential amino acids are synthes by *P. flavo-glaucum,* but that cystine is a limiting factor when are restricted to this mold as the sole source of protein. A r extensive study of the problem by Gorcica, Peterson and Steenb ('35) indicates that *Aspergillus sydowi* is of quite poor quality w fed as the only source of protein. However, whole wheat or gluten, fed at a low level, was able to confer a marked supplemen effect upon the mold. Undoubtedly the further study of micr ganisms, as sources of dietary essentials, will yield interesting valuable results.

Supplementary Relationships Between Proteins and Am Acids.—For reasons presented in Chapter V, practical dietetics animal husbandry are dependent, as respects protein nutrition, u the reinforcement of essential amino acid inadequacies in availa proteins with other available proteins which contain such amino a in relative abundance, but which, perhaps, are inadequate in th possessed in relative excess by the first. Thus an understanding supplementation is simple indeed.

As a rule animal proteins constitute good supplements for cer and vegetable proteins. Evidence of this fact was made available the extensive early studies of Osborne and Mendel and those McCollum and Simmonds. Most recent contributions have been m by Mitchell et al., and others.

Instructive data upon the supplementary relationships between b and vegetable proteins, for example, are furnished by Hoagland a Snider ('27) (Table III). When one part of beef protein was mi with two parts of vegetable protein, the rations containing the lowing vegetable products had approximately the same value inducing growth, viz., wheat, bolted wheat flour, corn meal, and

eal. Each of these mixtures also had approximately the same value r inducing growth as a ration containing the same percentage of f protein alone.

According to Mitchell and Carman's ('26, l.c.) nitrogen balance ta, milk, and white flour mixed (1 to 2) gave a value of 71, whereas e computed value, based on the nutritional value of each reckoned parately, was only 62. Moreover, white flour and beef (2 to 1) gave value of 73, whereas the computed value was about 64. Thus in ch instance a definite supplementary effect is indicated.

In certain countries, particularly the Orient, it would be of great gienic and economic importance if some combination of vegetable oteins could be found which supplement each other to the extent providing an amino acid mixture as satisfactory as that furnished combinations of animal and vegetable proteins. According to dolph and Cheng ('35) a flour made from a mixture of corn, millet, d soy beans, has a nutritional value considerably higher than that any of the three components alone. Moreover, this mixed cereal ur is said to occupy a prominent place in the Chinese dietary. How-er, it appears improbable that any combination of vegetable pro-ns can be found which has the marked supplementary relationships exists between animal and vegetable proteins. Vegetable proteins nd, almost invariably, to be somewhat deficient in lysine. And, as phasized by Mitchell and Smuts ('32), tryptophane tends to occur inadequate amounts, particularly in corn. In general, beef and soy an proteins are deficient in cystine, wheat protein in lysine, corn otein in lysine and tryptophane, and oat protein in lysine.

Protein Requirements.—A perennial problem is the question of otein requirements for growth, maintenance in adult life, preg-ncy, lactation, various conditions of disease, live stock production, lk and egg production, and even other departments of nutrition. evertheless, there is considerable basis for belief that good health n be maintained although the protein intake, provided it is of isfactory quality, varies considerably from the level which is opti-um for the body.

In the last century deductions of Voit, Atwater, and others, on the sis of statistical studies of human dietaries, placed the adult daily quirement for protein at about 110 to 120 gm. On the basis of esent evidence, obtained by experimental procedures, this value is obably somewhat near the average normal requirement. Thus in s particular case the results, secured by unphysiological methods of culation, appear to be approximately correct.

At the beginning of this century Chittenden conceived the idea at a low protein dietary will best meet the nutritive requirements of body. He emphasized the desirability of relieving the body of the

task of degrading more protein than is necessary for the repl of nitrogenous compounds used in metabolism and tissue rep principal deduction was that protein intake should be reduc low a level as possible, and still keep the body in nitrogen equi In the evaluation of this thesis numerous adults were observ a period of 9 months during which the protein intake was to about half the accepted requirements. Since they appeared excellent condition at the end of the period of observation concluded that such a dietary regimen appeared to be suita maintenance of well-being in the adult for an indefinite perio that time numerous advocates of low-protein regimens have themselves heard but convincing proof of such a policy, for application, has not been demonstrated.

Of the numerous experimental data of McCollum and ass none support this view. They all point to the conclusion generous protein ingestion which just suffices to induce m growth in the young serves to maintain vigor in adults for the l possible period. Since these studies were made twenty years ago, knowledge of dietary essentials was as advanced as it is now believed that further study of the question should be made, as criteria, longevity and general health throughout adulthood. ever, there is no reason to believe that further research, in the li present knowledge, would indicate a reversion to the thesis of tenden and others who advocated a low-protein regimen. The vig arguments of Christiansen ('34), Süsskind ('34), and others, a the low-dietary protein thesis of Hindhede ('34) and his suppo provide further evidence that, in general, the safest policy is to tain liberal amounts of protein in the diet.

As subsequent discussion will show, several types of diseas referable to inadequate protein intake. Even persons with c types of nephritis appear to be benefited by the maintenance o protein intake at a fairly liberal level (McCann, '31). As show Peters ('32), tuberculosis, diabetes, and some gastrointestinal di may be treated by the restoration of serum protein to a normal through an increase of protein in the diet.

Space does not permit a consideration of the protein nee various age groups and such special cases as pregnancy and lact The discussion of these subjects is more appropriate in special and monographs written for that purpose. In addition to brief ments it suffices to mention that the protein requirements of chi have been studied by numerous workers including Wang, Hawk Hays ('28), McQuarrie ('29), Parsons ('30), and Daniels et al. Daniels et al. conclude that children of preschool age should re approximately 3.2 gm. of protein per kilogram in diets furnishi

ast 50 per cent of the protein from animal sources. There seems be no very authoritative opinions regarding the protein needs of olescence. Undoubtedly the great variability in growth rate, as well other factors, during that period precludes the application of more an very general standards.

Slonaker's Observations on the Effects of Different Amounts Protein in the Diet.—As indicated above, it is of interest to de-mine the effects upon various life functions of different amounts protein in the diet. The studies of Slonaker ('31), although fortunately faulty in some important aspects, are sufficiently in-uctive to warrant some discussion. The five diets used in this study d the following composition, as calculated from Slonaker's data:

Components	Per Cent				
	I	II	III	IV	V
rnstarch	48.31	44.44	40.48	36.49	32.36
ole ground wheat	19.32	17.78	16.19	14.59	12.95
ole ground yellow corn	9.67	8.89	8.13	7.30	6.41
mmed milk powder	3.87	3.56	3.24	2.92	2.59
alfa leaf flour	3.87	3.56	3.24	2.92	2.59
nmercial casein	1.93	1.78	1.62	1.46	1.31
at scrap	0.96	8.89	17.00	25.18	33.66
eat germ	2.90	2.67	2.43	2.19	1.94
salted butter	4.83	4.44	4.05	3.65	3.24
ast	1.93	1.78	1.62	1.46	1.31
ium chloride	0.96	0.89	0.81	0.73	0.64
cium carbonate	1.45	1.33	1.21	1.09	0.97

In addition to the above table it is helpful to estimate the calcium d phosphorus content of the different diets. Although it is im-sible to make more than a very rough approximation, the errors uld be relatively constant. The table which follows was calculated the assumption that the calcium and phosphorus content of naker's dietary components approximated the values given in ndard tables on the composition of foods:

Diet I		Diet II		Diet III		Diet IV		Diet V	
a	P	Ca	P	Ca	P	Ca	P	Ca	P
n.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
14	0.2013	0.5917	0.2349	0.5459	0.2664	0.4977	0.3003	0.4534	0.3342

According to Slonaker's calculations, the protein content o diets was: 10.3; 14.2; 18.2; 22.2; and 26.3 per cent respectiv diets I to V inclusive. Some of the results of feeding these d rats throughout life, were as follows: The average life spa longest to shortest was in the order of Groups II, III, I, IV, During the first 120 days the average per cent increase in body from greatest to least was in the order of Groups V, IV, III, II, Group V averaged 306 per cent and I averaged 112 per cen order of maximal weight was Group III, 307; II, 294; I, 288; and IV, 247 gm. The average number of litters and the a number of young born, from greatest to least, was in the or Groups II, I, III, IV, and V. The size of the litters was in the of I, II, III, IV, and V.

On the basis of his numerous observations and calcul Slonaker concluded "that when best growth is considered the a of protein should be slightly in excess of 14 per cent." But wh method of preparing the diets is considered it is apparent that i of one variable, namely protein, there are at least eleven variable all of the various components of the diet! This was caused b unusual method used to construct the diets. Diet I was used basal ration. Diet II was made by adding 900 gm. of meat scr 10,350 gm. of diet I. Two thousand grams of meat scrap were to 10,350 gm. of diet II to make diet III, and so on. Thus procedure caused the inclusion of many variables, as is indicate the above table on the percentage composition of the diets. Fo ample, in diet I the amount of yeast is 1.93 per cent but in d it is only 1.31 per cent. Also, the amount of calcium carbonate i I is 1.45 per cent and it is only 0.97 per cent in diet V. Finally calcium: phosphorus ratio of diet I is much higher than that of di Since the content of vitamin D is almost nil it may be supposed the effect of diet I on calcium and phosphorus metabolism is different from that of diet V. Diets II, III, and IV occupy mediate positions in these relationships. Since all of the vari undeniably influence rate of growth, reproduction, longevity, etc. obvious that the results of these studies are practically meanin owing to the multiplicity of uncontrolled variables.

Moreover, meat scrap is not a pure protein. At best it repre a concentrated source of digestible protein, and at worst, ma which consists in great measure of connective tissue and cart It contains large and significant amounts of phosphorus as we other minerals, especially potassium.

These experiments, although practically impossible of interp tion, are somewhat typical of nearly all investigations concernin effects of various levels of protein in the diet. The general fa

t nearly all so-called proteins, as used in dietary studies, are at best
de materials containing significant amounts of substances which
not protein. For example, the most commonly used protein, casein,
tains almost one per cent of phosphorus. It is easy to imagine,
refore, that the results obtained with casein at a high level, perhaps
ber cent of the diet, as compared with this material at 15 per cent of
diet, are not open to interpretation only on the basis of a difference
rotein level in the diet; the differences in phosphorus must also be
koned if an accurate evaluation is to be made. In the past there
been, perhaps, some excuse for failure to consider some of these
gs in studies or proteins, owing to the lack of information con-
ning the "importance of little things," but in the future investi-
rs must pay stricter attention to the planning of their experiments
ney are to obtain significant information.

The Effect of Heat upon the Nutritional Value of Proteins.—
st proteins are profoundly changed in their physical properties by
g heated to their coagulation temperatures. The evidence available
nts to the nature of this change as one of polymerization. Attention
been called to the changes in the molecular weights of proteins
different points along the pH range (Chapter V). Chick and
tin ('10) have shown that water must be present when denatur-
n occurs. Also Wu and Wu ('25) offer evidence that some hydro-
splitting of the molecule occurs. They have discussed the views
ifferent investigators concerning the nature of denaturation, which
term loosely used to designate the change of proteins from a
ble to an insoluble form by heat, light, pressure, acids, alkalies,
ain organic solvents, salts of heavy metals, dyes, etc. We are here
rested only in the effect of heat treatment, which is widely applied
ne preparation of foods.

Many foods are subjected to more or less dry heat treatment, as in
ping corn, roasting peanuts, baking crackers, and the preparation
aked or puffed cereals. Frying foods or deep frying in fat may
lt in superheating of a part of the protein of a food. Pressure
kers, which are widely used, superheat the proteins. We should, of
se, carefully distinguish between heating with superheated
n, involving the exclusion of air, which would cause hydrolytic
rage of proteins, and dry heat treatment, where oxidation might
r.

Morgan ('30) conducted growth studies with young rats which
ved that the protein of cereals was not well utilized after dry
ng or toasting at approximately 200° F. for 45 minutes. Toasted
le wheat showed 15 per cent less absorbability than raw wheat
ein. The addition of 5 per cent of unheated casein very nearly
e up the discrepancy between the toasted and the raw diets,

showing that the heat treatment affected the protein rat another constituent of the diet.

When young rats were fed diets containing raw and toast F. for 30 minutes) wheat gluten as a source of protein at 18, 21, and 24 per cent levels, in an otherwise adequate diet, th in 56 days, per gram of protein eaten, was found to be 1.2 for raw gluten at levels up to 18 per cent, and to fall to 1.15 at 21 and 24 per cent respectively. The corresponding figure toasted gluten were 0.94 to 1.09, and 1.12 and 0.86. The m growth rate on both raw and toasted diets occurred at th cent level.

The nutritional values of raw, water-cooked, and toaste wheat protein, as determined by the nitrogen balance me Mitchell, were found to be 64, 67, and 52 respectively, and 67, 69 when supplemented by 5 per cent of unheated casein. In a comparison of the values of raw and toasted wheat gluten approximately 12 per cent level the figures obtained were 66 respectively. Large and mature rats, when fed raw and toaste gluten at 6 per cent level, gave nutritional values of 83 and when fed at a level of 8 per cent protein the values were 66 respectively.

The digestibility of the toasted proteins was but little d from that of the unheated, particularly in older animals, a unexplainable loss of nitrogen occurred chiefly in the urine, ind that the change produced by the heat treatment was proba assortment or availability of the amino acids absorbed. Cookin water had scarcely any effect on the nutritional values of protei caramel formation during toasting was found to account for small part of the injury.

Chick and her associates ('35) compared the nutritional va several proteins. They were the proteins of whole wheat, whit wheat germ, maize endosperm, whole milk, lactalbumin, lactalbumin (at 120° C. for 72 hours), casein, heated case 112-125° C. for 72 hours), and heated casein purified by reprecip and extraction with dilute alcohol. These were fed at levels from 3 to 10 per cent of the diet. The value of casein was not l by heat treatment at 112 to 125° for 72 hours or by further purifi Heating it to 150° for 66 hours decreased its value from 64 to per cent of protein in the diet), and reduced the digestibility 93 to 73 per cent. The heat-treated lactalbumin (120° for 72 was scarcely reduced in nutritional value. However, its diges was reduced from 95 to 69 per cent.

Seegers and Mattill ('35) observed essentially the same growth in rats fed adequate diets deriving almost all of their

5 per cent) from beef heart, kidney, round, and liver. Pork liver as equal to beef liver. When these tissues were continuously extracted during 60 hours with 95 per cent alcohol there was but little duction of nutritional value of the protein except in the case of er, the proteins of which were greatly altered by prolonged contact ith hot alcohol. When liver was extracted 130 hours it did not pport growth or life. It was shown to have but slight digestibility. ver heated at 100° C. for 14 days no longer supported growth though its digestibility and nutritional value were not lowered preciably. Above 100° C. there was progressive lowering of its gestibility. Heart and round were resistant to change in nutritive lue at 120° C., and kidney was still digestible after heating at 0° C. At higher temperatures these tissues also became refractory digestive enzymes. It is pointed out that these changes in digestility and in nutritive value do not occur to a significant degree in mestic cookery, but are of great importance in the preparation of rified proteins for nutrition studies.

Morgan and Kern ('34), however, found the value for maintenance raw beef muscle protein at 7 per cent level to be greater for rats an that of the same meat cooked in three different ways. The lue for raw beef, by the Mitchell method, is 67, for that boiled at dinary pressure (internal temperature 85° C.), 60; boiled for 7 inutes at 15 lbs. pressure (internal temperature 85° C.), 60; and iled for 1 hour at 15 lbs. pressure, 56. A heat injury appears to cur, which increases with length of exposure and intensity of heating.

That the depreciation of protein on heating is not solely one of ysical change which interferes with digestion, follows from the ct that the observed nutritional values fall with heat treatment, and so from the findings of Greaves and Morgan ('34), who supplented heat-treated casein with 0.2 per cent of lysine, histidine, rosine, cystine, and tryptophane respectively. Cystine addition inreased the rate of growth with both raw and heated casein, uniformly about 15 per cent above the unsupplemented values, but the fference between raw and heated protein still remained. It seems obable that cystine is not the damaged part of the molecule. Both ine and histidine supplements increased the value of the heated sein but did not affect that of the raw, as illustrated by their figures.

	Gain per Gram of Protein Eaten
Unsupplemented heated casein	1.76 grams
Lysine supplemented heated casein	1.89 "
Histidine supplemented heated casein	1.96 "
Lysine supplemented raw casein	2.00 "
Histidine supplemented raw casein	3.14 "
Unsupplemented raw casein	2.10 "

Tyrosine and tryptophane additions did not increase the v heated casein. It is concluded that heat treatment of casein i experiments caused change in the lysine and histidine units protein which destroyed their nutritive value.

Block, Jones and Gersdorff ('34) were stimulated by the of Greaves and Morgan to determine the lysine in casein whi been heated to 150° C. for 65 minutes. Their results show th treatment does not materially affect the content of this amin Their yield of lysine in unheated casein was 6.5 per cent, and two samples of heated casein was 5.5 and 6.6 per cent respe The results of Greaves and Morgan are, therefore, not to be exp on the basis of existing data.

Hayward, Steenbock and Bohstedt ('36b) observed effects contrary to the above described deterioration of nutritive va proteins from heat treatment, in their studies of soy bean pr They cite several investigations which show that ground raw soy when fed with the necessary supplements, as the sole or pri source of protein, do not support growth. Normal growth re when the beans were cooked. Osborne and Mendel ('17) and and Shrewsbury ('32) reported that cooking soy beans increase digestibility of their protein by about 4 per cent. They both that the improved nutritive value of these beans caused by he appeared to be due to an increase in food consumption and nitr absorption.

Johns and Finks ('20) found that phaseolin, an isolated pr from the navy bean, produced markedly better growth when co than when raw. A similar result was observed with the iso proteins of the velvet bean (Finks and Johns, '21). Both of proteins were better digested *in vitro* after cooking than when The results of Hayward et al., obtained by means of the Osb Mendel and Ferry method, are summarized as follows:

Soy Beans	*Growth per Gram of Protein Eaten*
Raw	0.93 (32% of diet)
Heated in electric oven at 135° C. 1.5 hr.	0.48 (18% of diet)
Heated in sealed bomb at 125° C. 1.5 hr.	0.93 (18% of diet)
Autoclaved at 125° C. 1.5 hr.	1.22 (18% of diet)
Raw, oil extracted with ether at 25° C.	0.31 (18% of diet)

By the balance sheet method they found the average dige coefficients of raw and heated soy beans in six experiments to b and 87 per cent respectively, and the average nutritional values 41 51 respectively. Commercial soy bean meals from which the oil been expressed at temperatures of 112 to 130, and 140 to 150 during 2.5 minutes, or hydraulic meals cooked at 105 and 121° C

minutes, as in the commercial solvent method of oil extraction, as an effective method of heat treatment. Efficient heat treatment anges the meal from light brown to brown color, and the color may used as an index to quality of protein in these meals, but only in e product of the expeller and hydraulic processes.

The food intake during the first few days of all rats in these periments was similar when raw or heated meal was fed. This ggests that the poor growth on raw bean meal, and low temperature eal, was due to some deficiency in the proteins rather than to a lack palatability. When casein was added as a supplement to raw soy ans, normal growth resulted. This is evidence that the deficiency s in the protein fraction. Heating raw soy beans to the higher mperatures reached in commercial processing increased the digesti- lity by about 3 per cent, and the nutritional value by about 12 r cent.

Effect of Ultraviolet Irradiation on the Nutritive Value of oteins.—Koch and Koch ('32) state that irradiation of casein and egg white, in the dry state, slightly reduces their digestibility by psin and trypsin *in vitro*. Metabolism experiments on young rats, the other hand, demonstrate a slightly higher growth rate when e protein is irradiated. The nitrogen retention on their low protein ion was slightly less for the irradiated than on the non-irradiated otein. Hence the increase in growth is due to some factor other an improved utilization of protein. Ample vitamin D was provided both experiments, so the stimulation of growth is attributable to me other effect which is unknown at present. Scarcely anything ore is known about this relationship. It should be further vestigated.

The Nutritional Effect of Deaminized Protein.—When a protein treated with nitrous acid deamination of its free amino groups curs. This results in extensive mutilation of the protein as shown Van Slyke and Birchard ('13-14), who demonstrated that casein es half its lysine when deaminized in this manner. But Wiley and wis ('30) claim that in deaminized casein all the lysine is destroyed, lf the histidine is lost, tyrosine is partially destroyed, and arginine d tryptophane are unchanged. According to White ('33) cystine duction does not occur with deamination.

It appears that in addition to the very poor nutritional value, a iking effect of deaminized casein is the anemia which results from inclusion in the diet of rats even at a level as low as 5 to 10 per nt. This was first demonstrated by Hogan and Ritchie ('34). latin and gliadin together were an adequate source of protein, but nen deaminized casein was added to the mixture the animals became emic, failed to grow, and died. Although milk, egg yolk, wheat

germ oil, ventriculin, muscle, liver, stomach, laboratory-
lactalbumin, autoclaved casein, and autoclaved yeast, are ineff
preventing the anemia, commercial preparations of lactalbu
treated casein, and dried yeast are protective against the
(Hogan, Guerrant and Ritchie, '36).

The evidence suggests that deaminized casein contains so
which causes anemia. Some elucidation of the question was fu
by Smith and Stohlman ('36), who showed that the deaminize
produced in the rat a characteristic macrocytic megaloblastic
with many Howell-Jollie bodies. They affirm that supplement
the deaminized casein with good quality protein does not prev
pathological process, though it reduces the severity of the
That the anemia is an intoxication, rather than a deficiency
is indicated by Smith and Stohlman's observation that the an
reproduced by the intraperitoneal injection of a hydrolysate
of deaminized casein. Whether other proteins are similarly affe
deaminization is not known.

High Protein Diets and Renal Damage.—The question w
kidney injury may result from the ingestion of large amou
protein has been studied by various investigators. Newburgh
observed that rabbit's kidneys were injured by a high protein d
Squier and Newburgh ('21) reported that human kidneys were
aged by diets containing a high content of beef protein. New
and Clarkson ('23) fed rabbits 26.8 and 36.2 per cent of protei
found kidney lesions. In the belief that the rabbit, being an h
orous animal, and accustomed to succulent vegetable foods o
protein content, might be less tolerant of a high protein diet th
Omnivora or Carnivora, Polvogt, McCollum and Simmonds ('23
ducted comparable experiments with rats, using diets containi
to 41.3 per cent of protein. They observed hyalinization i
glomeruli, and hyaline material in the convoluted tubules,
adhesions between glomerulus and capsule in the Malpighian
There was also some infiltration and congestion surroundi
capsules.

Then followed several investigations by others, who failed t
firm these results. Osborne and Mendel ('24-25), Osborne, M
Park and Winternitz ('25), and Miller ('25), reported marked
trophy of the kidneys, but no abnormalities in histological str
in rats fed excessive amounts of protein. Evans and Risley
confirmed the early observations, but Anderson ('26), Jackso
Riggs ('26), Smith and Moise ('27), and MacKay, MacKay and
('24) reported hypertrophy without lesions.

It is possible that these conflicting researches might be exp
at least in so far as hypertrophy is concerned, on the basis

creased requirement for thiamin when high levels of protein are fed. ch a relationship was suggested by Drummond, Crowden and Hill 2); by Reader and Drummond ('24-25); Hartwell ('24); and quhart, MacLean and Smith ('26). The last named investigators d not find kidney lesions when rabbits receiving 60 per cent of otein were given green food every day. But upon withdrawal of s supplement renal injury developed as evidenced by albumin and ts in the urine. Hassan and Drummond ('27) noted such a relation- p, and Hartwell ('28) observed that the protective substance was t destroyed by autoclaving. Her findings suggest that it is not amin which prevents the renal injury. Since the rabbit synthesizes orbic acid, the effect of green food is not attributable to deficiency this factor. Cox, Smythe and Fishback ('29), using rats, observed inite tubular lesions when 0.3, 0.6, and 0.9 per cent of cystine was ded to the diet. The lesions were called "acute toxic nephrosis." x et al. found that the kidney injury did not occur when cystine s fed and the diet was supplemented by the daily addition of 300 . of the Osborne and Wakeman yeast concentrate (B complex). Longwell, Hill and Lewis ('32) studied this problem, and con- ded that vitamin B complex in the diet prevents hypertrophy of the dneys of the rat under the influence of much cystine or excess of ein. Cystine fed at 0.3 per cent of the diet causes no greater degree renal hypertrophy than the absence of yeast extract from an other- e normal diet. Feeding 0.6 per cent of cystine, however, causes pertrophy which may be prevented by the administration of the amin B complex. Similar results are observed when high casein ding is the cause of renal hypertrophy. Graded doses of the B nplex from yeast causes a progressively decreasing response of the neys to the administration of cystine, up to a certain point, above ich increase does not have a further beneficial effect. According these findings cystine is not directly nephrotoxic to rats, but it does, the absence of some water-soluble vitamin, cause renal hypertrophy. wever, one must conclude that the relation of dietary protein level kidney lesions remains a mystery.

Tissue Injury Caused by Amino Acid Imbalance.—The indica- s that excessive protein ingestion causes renal injury, particularly en certain proteins are used, suggest that amino acid imbalance ght be involved. The work of Newburgh and Curtis ('28), espe- ly, indicates this relationship. For instance, a diet containing 75 per t of dried liver produced a granular kidney in less than one year, the same amount of casein, fed 16 months, caused only moderate ular injury. It is not improbable, of course, that other factors ociated with the proteins were etiological factors in renal damage. example, both liver and casein contain large amounts of phos-

phorus which, if fed in excess, injures the kidneys and other o
However, amino acids alone are harmful when ingested in
amounts. Newburgh and Marsh ('25) observed definite nephrot
in rabbits fed arginine, aspartic acid, histidine, lysine, tyrosine, t
phane, and cystine. Also, Lewis ('25) found cystine to be nephr

Striking evidence of the relationship of amino acid imbala
renal and hepatic injury is furnished by the studies of Sullivan,
and Sebrell ('31) and Lillie ('32), in addition to that of some
workers. For example, a basal diet containing approximatel
maintenance level of protein, casein, at 4 per cent, was supplem
with various amounts of pure amino acids. When fed at the
cent level, tyrosine was decidedly toxic, more so than cystine at
level. Glutathione at a 25 per cent level, equivalent to 10 per
of cystine, was less toxic than 10 per cent of cystine. Also, 10 per
of tyrosine and 5 per cent of cystine was less toxic than 10 per
of tyrosine alone. The injury caused by tyrosine was strikingly d
ent from that caused by cystine.

These data suggest that the injuries related to proteins and a
acids are less severe if the amino acids are ingested as protein,
the amino acids are in certain proportions. They raise the que
whether certain proteins which yield some amino acids in very l
or small amounts might be injurious if they constitute the prin
source of amino acids in the diet. They also increase interest in
problem of the proper range of protein level in the diet and the ef
of excessive protein intake.

Edema Caused by Protein Deficiency.—Edema has been o
observed in persons suffering from beriberi. In the dry form of
disease there is usually emaciation; in the wet form edema complic
thiamin deficiency. A history of epidemics of edema associated
beriberi is given by Hirsch (1885). Under conditions of war, of pri
and of famine, edema has frequently occurred in epidemic incide
It has been variously spoken of as wet beriberi, war dropsy, hu
swelling, etc. The subsidence of edema in human subjects upon
provement of the diet has been many times observed, but the na
of the etiological factor concerned remained quite unknown u
after edema was produced experimentally in animals by restrictio
the protein intake. Studies of this type opened the way to impor
observations on edema in man associated with protein deprivation,
there are some points the nature of which is still obscure.

Denton and Kohman ('18), while studying the dietary proper
of carrots, observed that some of their animals, when restricted
carrots alone, or carrots with certain nutrient supplements, develo
edema. Later Maver ('19) and Kohman ('20) found that edema co
be produced in rats by restricting them to carrots, cornstarch, lard,

s, for periods of 8 to 12 weeks. They made various additions to this t, and found that only by increasing its protein content could the ma be alleviated. Frisch, Mendel and Peters ('29) repeated and ended these studies. They employed diets of the following nposition:

	Wet Diet	*Dry Diet*
Carrots	4500 gm.	550 gm.
Starch	360 gm.	360 gm.
Lard	60 gm.	60 gm.
Salt mixture (McCollum and Davis, J. Biol. Chem., *21*, 615, 1915)	35.6 gm.	35.6 gm.
Proteins	0.7 per cent	6.3 per cent
Fats	1.6 per cent	7.0 per cent
Carbohydrates	15.5 per cent	68.8 per cent
Salts	1.4 per cent	6.3 per cent
Water	80.0 per cent	13.0 per cent

Rats developed edema on both the wet and the dry diet, as nman had observed. The inclusion of 5 per cent of casein in the diet prevented the development of edema. There was general sarca, varying in degree. There was practically no gross fat visible the muscles were small and pale. Some of the animals died of g infections. Serum proteins showed a marked decrease in almost y case after two to three months, the reduction amounting to ut 40 per cent. That reduction of serum protein was the important or is supported by the work of Leiter ('28) who centrifuged off plasma of dogs, mixed the cells with normal saline, and reintro-ed them into the same animals. The serum proteins were reduced edema developed. Darrow, Hopper and Cary ('32) confirmed e observations with human infants who were being fed flour or and water because of digestive disturbances, and who developed na. Butter, salt or sugar failed to relieve the condition, but the nts improved rapidly when protein was added to the food, or n blood transfusions were given. Frisch, Mendel and Peters ted out that serum protein reduction is only partly responsible the production of edema. "Here, as in other conditions, they rmine a tendency toward water retention which is influenced by r factors. The fluid intake is very important, for if it is reduced ny way . . . no edema develops, even if the serum proteins are ally quite low."

Cutting and Cutter ('34-35) placed 26 normal human subjects for days on a diet containing only 500 calories per day, and no ein. Fluids were not restricted. Curiously, instead of falling, the serum protein concentration rose during the test. The concentra-of blood and of serum protein was attributed principally to low intake. Two days of protein deprivation is an insufficient time ake any significant change in the character of the blood proteins,

but the concentration is definitely lowered. This observat harmony with that of Torbert ('35), who showed that st lose a small percentage of protein of the plasma in 4 to 6 remain at a constant reduced level until death. The lost principally albumin.

Weech, Snelling and Goettsch ('33) studied the edema in dogs by restriction of the protein in the diet. When edem sidered as a degree of interstitial fluid accumulation great e permit detection by finger palpation, a fairly close correlation between this symptom and the level of plasma albumin. W centrations less than 1 gm. per 100 cc. of plasma edema i present; with concentrations above 2 gm. per 100 cc. it is near absent. The subcutaneous edema fluid from dogs with nu edema contains in most instances between 0.10 and 0.35 gm. o per 100 cc. Plasma specific gravity follows the fluctuations plasma protein and does not exhibit a correlation with edem ence has already been made to the studies of Block (Cha which show that the albumin of blood plasma is not a distinct but is variable in amount and composition depending u method of preparation.

Shelburne ('34) has produced edema in dogs by prolonged tion to a low protein diet. He found the critical level of proteins for the production of edema to be 4 gm. of total prot 2 gm. of plasma albumin per 100 cc. He observed fatty change renal tubules in all of his edematous animals, and offers evide these changes were not caused by the accompanying anemia invariably complicates such experiments.

Youmans and his associates ('32) have recorded the clinic ings of a group of patients whose principal complaint was mild apparently of nutritional origin. The diet of 12 of these was before the beginning of treatment, and in 11 cases it was fou the total caloric intake was below the basal requirement. The protein ingested ranged from 20 to 52 gm. daily, and was be minimum requirement in all but four cases. The minimum ously estimated to be from 0.5 to 0.7 gm. per kg. of weig refers the edema to chronic dietary deficiency, particularly to s of protein.

Lin and his associates ('32) studied two patients with nu edema. Edema was reproduced or accentuated twice by plac patients on a protein-free diet, with accompanying significant in the plasma proteins. A vegetable diet fed at a level of 1 protein per kg. of weight, and 7 per cent of total caloric inta not sufficient to establish a positive nitrogen balance, and to the plasma protein to the point of disappearance of edema. Tw

vegetable protein sufficed for this purpose. This corresponded to 14 cent of the caloric intake. Animal protein at a level of 7 per cent the caloric intake was effective in restoring the plasma protein l in relieving the edema. The digestibility and nutritional value of ir animal protein determined by the Mitchell method was much erior to the vegetable protein.

The subject of dietary protein in relation to edema is still in need further investigation. Bloomfield ('33) has called in question the erimental work of Frisch, Mendel and Peters, by pointing out that repeated withdrawal of blood for analysis may have played a t in the lowering of plasma protein. In rats kept on a low protein t during 21 weeks he found no significant decrease in serum tein concentration aside from an initial drop. His data are, in cral, supported by those of Weech, Goettsch and Reeves ('35), o include in their paper a good discussion of dietary protein and oproteinemia.

Dietary Protein and Plasma Protein Regeneration.—Several re-ches have provided data concerning the value of the proteins from ferent sources for plasma protein regeneration. McNaught and his ciates ('36) have studied this problem, using dogs whose plasma teins were kept at a low level, i.e., about 4 per cent, by means of eated plasmapheresis and a low-protein diet. The basis of evaluat- the proteins was the increased plasmapheresis needed to maintain standard level of plasma protein when different sources of protein e added to the diet. They expressed the power of the food protein yield plasma protein by the number of grams of food protein uired to form 1 gram of plasma protein.

They reported that the protein of potato, bran, rice, and rice shings have a higher value than animal proteins such as liver, n, or spleen. Those of the soy bean were especially effective. Most nt proteins favored the production of globulin, but those of soy ns resembled the animal proteins in favoring the formation of umin. The potency ratios, or grams of food protein required for eneration of one gram of blood proteins, as determined by Naught et al., are shown in the table on page 148.

McNaught and associates found that infection and intoxication urb the plasma protein production of standardized dogs and may uce the output of plasma proteins to very low levels in spite of siderable protein intake. There may be a very sharp drop in the tein level during the first day of intoxication.

Cartland and Koch ('28) state that diets deficient in proteins, mins, or minerals delayed regeneration of blood in rats made mic by bleeding. Koessler and his associates ('26) claim that blood eneration cannot take place in vitamin A deficiency, and that the

Source of Protein in Food	*Potency Rat*
Beef serum	2.6
Wheat protein (60 per cent)	4.2
Bran	4.3
Potato-bran, basal ration	5.5, 4.8, 4.1, 2.7, 4.2
Rice polishings	5.3
Gizzard	5.3
Kidney basal ration	5.3, 5.3*
Rice basal ration	5.4
Lactalbumin	5.5
Skeletal muscle	5.7
Egg white	5.8
Irish potato	6.1
Liver	6.4, 6.5, 6.8*
Soy bean	4.6, 7.1*
Liver residue	7.4
Beef heart	8.0
Liver extract	8.6
Casein	10.0
Spleen	10.2
Brain	11.8
Stomach	13.6
Salmon	15.2
Pancreas	19.0
Kidney	20.8

* Values obtained in different experiments.

rate of regeneration is a function of the amount of vitamin A able. Whipple and his associates ('28), as a result of nu studies, conclude that many tissues and fruits, especially a when fed to dogs stimulate hemoglobin regeneration, and tha exists some unknown substance which aids in the utilizat proteins in the synthesis of hemoglobin. Saunders ('26) foun when young rats were restricted to wheat gluten as the sole of protein, they became anemic, whereas when given 10 to 18 p of casein a high concentration of hemoglobin was produced. were able, on the casein diet, to regenerate their blood volum pletely every 8 to 15 days. Feeding red corpuscles does not ap stimulate the rate of hemoglobin regeneration (Cartland and These investigators observed, contrary to Koessler et al., that not become anemic when fed diets deficient in vitamins A, B

Madden and coworkers ('37) suggest that plasma can con protein readily to body tissues or body stores, but only in amounts and with difficulty can the body tissues contribute to form blood plasma as, for example, in fasting. A sterile (turpentine) induces a marked reduction in plasma protein re tion, even when the animals are consuming an ample diet. quantities of live yeast upset digestion and interfere with formation, whereas autoclaved yeast is well utilized, and has potency ratio (4.4).

The studies which have been reported here clearly show tha

tein regeneration is easily upset by faulty nutrition, and especially a low protein intake. There appear to be great differences in the ue of proteins for this function. The biological values of food teins for this purpose are apparently quite different from those growth of the tissues. The significance of this consideration is gnized and emphasized by Melnick, Cowgill and Burack ('36), se recent studies of diet in the regeneration of plasma protein are d on well-planned technical procedures.

The Relation of Low-Protein Diets to the Etiology of Ulcer.—ments ('34) states that natives of the Island of Manus, New land, who eat a well-balanced diet rich in protein and vitamins are mune to tropical ulcer. Other natives who subsist upon a less well-nced diet in which the ratio of carbohydrate to protein is high, the supply of vitamins is poor, are susceptible. The highest dence was found among natives existing upon a diet composed ely of sago, rich in carbohydrate, and deficient in vitamins. He ulates that imperfect diet induces changes in the skin epithelium, king it a suitable medium for the growth of fusiform bacilli and ochaetes, which, upon entering a wound, rapidly convert it into an r. The incidence, which ranged up to 15 per cent of the population ertain villages, fell when the diet was supplemented with fish or . There was complete absence of ulcers in fishing villages where diet contained 60 to 70 per cent of protein. Clements suggests deficiency of vitamins A and B-complex may play a role as well rotein deficiency.

Hoelzel and DaCosta ('31) studied the effect of the major vitamin ciencies, of high and low carbohydrate, of fat, of protein, and of ous amounts and kinds of roughage in the diet, as possible logical factors in ulcers of the forestomach. They conclude that ration can be produced in the forestomach by simple protein riction. An exclusive diet of bran was eaten by the rats in enormous unts but no ulcers developed although the animals starved to h. Hence, malnutrition or inanition does not cause ulcers. The cts of bran were attributed in part to its acid-binding power, and part to the diluting effect of its bulk. These investigators believe the 61 per cent incidence of gastric ulcer in rats observed by penheimer and Larimore ('24), when the animals were restricted diet containing 95 per cent patent flour, was due to protein riction rather than to ingestion of hair as suggested by Pappen-ner. The suggestion is offered that ulcers may occur as a result ck of acid-binding power of a diet poor in protein.

The indicated relationship between low-protein intake and gastric rs is further supported by the observations of Sharpless ('37) that on a purified diet invariably developed squamous epithelium

hyperplasia of the forestomach when the only protein wa yeast at 4 and 5 per cent levels respectively. Neither vita B-complex seemed to be involved and the type of carboh was not significant. Neither cysteine hydrochloride nor prevented the lesion but cystine at a 0.2 per cent leve complete protection from injury. When the content of trebled lesions never occurred.

In view of the present slight evidence one is at a loss the mechanism of this relationship. Methionine and pe amino acids should be studied with respect to their ability the stomach injury. Certainly the general question of diet in relation to gastric ulcers should be further investigated.

Toxicity of Egg White.—Boas ('27) reported a nove in rats which were fed Chinese dried egg white. She charac condition as "eczematous dermatitis," accompanied by blepharitis, spasticity, and in some cases by edema of Occasionally there were skin hemorrhages. Attention was c similarity of some of the conditions to those seen in pellag and Goodman ('34), using a similar basal diet containin white, noted fur-like or woolly hair, alopecia, exfoliating hyperemia, skin hemorrhages, blepharitis, stomatitis, saliva able edema, erythema of the feet, symptoms of nervous d hypochlorhydria, and some anemia. Both fresh and dried caused the condition when fed in the raw form. Diets con more than 18 per cent of protein from this source cause the They state that the skin lesions were more severe on lov than on diets containing 18 per cent of butter fat or hyd cottonseed oil or linseed oil.

Salmon and Goodman found that when 18 per cent o white was fed the symptoms were prevented by the in brewer's yeast, dried liver, the extracted residue of yeast or not by extracted casein, extract or hydrolyzed residue o yeast, white corn, or baker's yeast. Liver extract was mor than yeast extract, but was only partially preventive. Well cases were cured by brewer's yeast, dried liver, milk, or residue of brewer's yeast, or dilute hydrochloric acid. Coag fresh egg white by heat and extraction of the coagulum w cent alcohol rendered it innocuous. The concentrated extra white had a slightly harmful effect but failed to produce symptoms caused by raw egg white. Salmon and Goodman their data to indicate the presence in raw egg white of harmful factor which is antagonized by the protective rather than the existence of a deficiency.

Parsons and Lease ('34) have made extensive studies on

rome. They point out that while the symptoms are strikingly similar those due to "vitamin G" deficiency, the provision of a great undance of this factor does not prevent the egg white injury. No udies have been made with nicotinic acid, riboflavin, or vitamin B_6 dividually. They have shown that the injury is not due to amino d deficiency since 30 per cent of dried beef heart or 60 per cent of ed milk in the diets containing raw egg white failed to effect a re of the dermatitis. Cooked pork kidney was the most effective bstance studied for preventing the egg white injury. One-sixth of e weight of egg white fed, in the form of pork kidney, sufficed for otection. Cooked beef liver and kidney and cooked pork liver are od sources of the protective substance. One to three times the ight of egg white, as dried yeast, dried egg yolk, wheat embryo, dried milk must be added to effect a cure. Spleen, heart, ovary, renal, blood, or hemoglobin, have little or no protective property. he activity resides in the residue after the preparation of Eli Lilly Company liver extract No. 343, but not in the extract. Prolonged ating at 100° C is destructive, especially if the material is dry. oiling with hydrochloric acid of 5 per cent or more concentration r 1 hour decreases the potency appreciably. They found that low moglobin is not a feature of the syndrome due to feeding egg white. ey conclude that the injury is not due to destruction by egg white some dietary factor in the ration.

Balls and Swenson ('34) conclude that egg white injury may be result of its anti-tryptic activity. The proteinase inhibitor is found the thin or watery fraction of egg white, whereas the thick white is initely proteolytic. They separated these two fractions and described procedure for concentrating the trypsin-inhibiting substance in the n white, one gram of active material being obtained from 20 gm. thin egg white. Parsons ('36) has employed such concentrates in ther studies, and found that these showed no corresponding increase the capacity of egg white rations to produce the nutritional disorder, on the other hand, permitted the healing of the characteristic ons when the diets incorporating them held a much higher con- tration of anti-trypsin than did the physiologically injurious diets taining the egg white or extracted residues. She concludes, there- e, that the egg white syndrome is not attributable to the anti-tryptic tent of this substance. The problem is still unsolved.

REFERENCES

McCollum and Steenbock (1912). Wis. Agric. Exper. Sta. Research Bull. No. 21, p. 69.

Mitchell and Hamilton (1929). "The Biochemistry of the A
The Chemical Catalog Co., New York.
Boas Fixsen (1934-35). Nutr. Abstr. & Rev., *4*, 447.
Mitchell (1924). Physiol. Rev., *4*, 424.
Chick and Roscoe (1930). Biochem. J., *24*, 1780.
Boas Fixsen and Jackson (1932). Biochem. J., *26*, 1919.
Mitchell (1926). Bull. Nat'l Research Council, Part 1, No.
Seegers and Mattill (1935). J. Biol. Chem., *110*, 531.
Osborne, Mendel and Ferry (1919). J. Biol. Chem., *37*, 223
McCollum and Simmonds (1929). "The Newer Knowledge o
4th ed., The Macmillan Co., New York.
Mitchell and Beadles (1929-30). J. Nutr., *2*, 225.
McCollum, Simmonds and Parsons (1921). J. Biol. Chem.,
175, 207, 235.
Hayward, Steenbock and Bohstedt (1936a). J. Nutr., *12*,
Funnell, McC., Vahlteich, Morris, MacLeod and Rose (193
11, 37.
Adolph and Wu (1934). J. Nutr., *7*, 381.
Rehfuss (1934). J. Am. Med. Assoc., *103*, 1600.
Shay, Gershon-Cohen and Fels (1936). Am. J. Digest. Dist.
235.
Mitchell (1923-24a). J. Biol. Chem., *58*, 923.
Mitchell, Beadles and Kruger (1927). J. Biol. Chem., *73*,
Hoagland and Snider (1936). J. Agric. Research, *53*, 349.
American Medical Association, Council on Foods (1936). J.
Assoc., *107*, 2132.
Mitchell and Carman (1926). J. Biol. Chem., *68*, 183.
Boas Fixsen, Hutchinson and Jackson (1934). Biochem. J.,
Mitchell (1923-24b). J. Biol. Chem., *58*, 905.
Mattill (1930). J. Nutr., *3*, 17.
Skinner (1934). J. Bact., *28*, 95.
Gorcica, Peterson and Steenbock (1935). J. Nutr., *9*, 701.
Hoagland and Snider (1927). J. Agric. Research, *34*, 297.
Adolph and Cheng (1935). Chinese J. Physiol., *9*, 245.
Mitchell and Smuts (1932). J. Biol. Chem., *95*, 263.
Christiansen (1934). München. med. Wchnschr., *81*, 1548.
Süsskind (1934). München. med. Wchnschr., *81*, 723.
Hindhede (1934). München. med. Wchnschr., *81*, 986.
McCann (1931). Ann. Internal Med., *5*, 579.
Peters, Bruckman, Eisenman, Hald and Wakeman (1932),
vestig., *11*, 97, 103.
Wang, Hawks and Hays (1928). Am. J. Dis. Child., *35*, 968.
McQuarrie (1929). J. Nutr., *2*, 31.
Parsons (1930). Am. J. Dis. Child., *39*, 1221.
Daniels, Hutton, Knott, Wright, Everson and Scoular (193
9, 91.
Slonaker (1931). Am. J. Physiol., *96*, 547, 557; ibid., *97*, 15, 32
ibid., *98*, 266.

HCK and MARTIN (1910). J. Physiol., *40*, 404.
U and WU (1925). J. Biol. Chem., *64*, 369.
ORGAN (1930). J. Biol. Chem., *90*, 771.
H CK, BOAS FIXSEN, HUTCHINSON and JACKSON (1935). Biochem. J., *29*, 1712.
EGERS and MATTILL (1935). J. Nutr., *10*, 271.
ORGAN and KERN (1934). J. Nutr., *7*, 367.
EAVES and MORGAN (1934). Proc. Soc. Exper. Biol. & Med., *31*, 506.
OCK, JONES and GERSDORFF (1934). J. Biol. Chem., *105*, 667.
AYWARD, STEENBOCK and BOHSTEDT (1936b). J. Nutr., *11*, 219.
BORNE and MENDEL (1917). J. Biol. Chem., *32*, 369.
STAL and SHREWSBURY (1932). Am. Soc. Animal Prod. Proc., p. 127.
HNS and FINKS (1920). J. Biol. Chem., *41*, 379.
NKS and JOHNS (1921). Am. J. Physiol., *57*, 61.
CH and KOCH (1932). Proc. Soc. Exper. Biol. & Med., *29*, 769.
N SLYKE and BIRCHARD (1913-14). J. Biol. Chem., *16*, 539.
LEY and LEWIS (1930). J. Biol. Chem., *86*, 511.
HITE (1933). J. Biol. Chem., *103*, 295.
GAN and RITCHIE (1934). J. Biol. Chem., *107*, 179.
GAN, GUERRANT and RITCHIE (1936). J. Biol. Chem., *115*, 659.
ITH and STOHLMAN (1936). U. S. Pub. Health Reports, *51*, 772.
WBURGH (1919). Arch. Internal Med., *24*, 259.
TIER and NEWBURGH (1921). Arch. Internal Med., *28*, 1.
WBURGH and CLARKSON (1923). Arch. Internal Med., *31*, 653.
LVOGT, MCCOLLUM and SIMMONDS (1923). Bull. Johns Hopkins Hosp., *4*, 168.
BORNE and MENDEL (1924-25). J. Biol. Chem., *59*, 339.
ORNE, MENDEL, PARK and WINTERNITZ (1925). Am. J. Physiol., *72*, 222.
LLER (1925). J. Exper. Med., *42*, 897.
NS and RISLEY (1925). Calif. & West. Med., *23*, 437.
DERSON (1926). Arch. Internal Med., *37*, 313.
KSON and RIGGS (1926). J. Biol. Chem., *67*, 101.
TH and MOISE (1927). J. Exper. Med., *45*, 263.
KAY, MACKAY and ADDIS (1924). J. Clin. Investig., *1*, 576; Proc. Soc. Exper. Biol. & Med., *24*, 335.
MMOND, CROWDEN and HILL (1922). J. Physiol., *56*, 413.
DER and DRUMMOND (1924-25). J. Physiol., *59*, 472; Biochem. J., *20*, 256.
TWELL (1924). Biochem. J., *18*, 785; ibid., (1925), *19*, 1075.
CHART, MACLEAN and SMITH (1926). Brit. J. Exper. Path., *7*, 360.
AN and DRUMMOND (1927). Biochem. J., *21*, 653.
TWELL (1928). Biochem. J., *22*, 1212.
. SMYTHE and FISHBACK (1929). J. Biol. Chem., *82*, 95; Cox and Hudn (1930). J. Nutr., *2*, 271.
WELL, HILL and LEWIS (1932). J. Nutr., *5*, 539.
BURGH and CURTIS (1928). Arch. Internal Med., *42*, 801.
BURGH and MARSH (1925). Arch. Internal Med., *36*, 682.
IS (1925). J. Biol. Chem., *65*, 187.

Sullivan, Hess and Sebrell (1931). J. Biol. Chem., *92*, lxvii.
Lillie (1932). U. S. Pub. Health Reports, *47*, 83.
Hirsch (1885). "Handbook of Geographical and Historical Path
vol. II, The New Sydenham Society, London.
Denton and Kohman (1918). J. Biol. Chem., *36*, 249.
Maver (1919). J. Am. Med. Assoc., *74*, 934.
Kohman (1920). Am. J. Physiol., *51*, 378.
Frisch, Mendel and Peters (1929). J. Biol. Chem., *84*, 167.
Leiter (1928). Proc. Soc. Exper. Biol. & Med., *26*, 173.
Darrow, Hopper and Cary (1932). J. Clin. Investig., *11*, 683.
Cutting and Cutter (1934-35). Proc. Soc. Exper. Biol. & Med., *32*,
Torbert (1935). J. Exper. Med., *62*, 1.
Weech, Snelling and Goettsch (1933). J. Clin. Investig., *12*, 193.
Shelburne (1934). Arch. Path., *17*, 152.
Youmans, Bell, Donley and Frank (1932). Arch. Internal Med., *50*
Lin, Chu, Wang and Chung (1932). Chinese J. Physiol., *6*, 73.
Bloomfield (1933). J. Exper. Med., *57*, 705.
Weech, Goettsch and Reeves (1935). J. Exper. Med., *61*, 299.
McNaught, Scott, Woods and Whipple (1936). J. Exper. Med., *63*,
Cartland and Koch (1928). Am. J. Physiol., *87*, 249.
Koessler, Maurer and Loughlin (1926). J. Am. Med. Assoc., *87*, 476
Whipple (1928). J. Am. Med. Assoc., *91*, 863; Whipple and Robs
Robbins (1925); Am. J. Physiol., *72*, 395, 408, 419, 431; ibid. (1927)
260, 271, 280; ibid. (1927), *80*, 391, 400.
Saunders (1926). Proc. Soc. Exper. Biol. & Med., *23*, 788.
Madden, Winslow, Howland and Whipple (1937). J. Exper. Med.
431.
Melnick, Cowgill and Burack (1936). J. Exper. Med., *64*, 897.
Clements (1934). Med. J. Australia, *1*, 520; J. Am. Med. Assoc. (19
106, 139.
Hoelzel and DaCosta (1931). Proc. Soc. Exper. Biol. & Med., *29*, 382
Pappenheimer and Larimore (1924). J. Exper. Med., *40*, 719.
Sharpless (1937). J. Nutr., *13*, Suppl., 19.
Boas (1927). Biochem. J., *21*, 712.
Salmon and Goodman (1934). J. Nutr., *8*, 1.
Parsons and Lease (1934). J. Nutr., *8*, 57; Biochem. J. (1934), *28*,
Balls and Swenson (1934). J. Biol. Chem., *106*, 409.
Parsons (1936). J. Biol. Chem., *116*, 685.

CHAPTER

VII

Calcium, Phosphorus, and Magnesium

NCE calcium and phosphorus constitute the principal mineral nponents of bone it must have been assumed several centuries ago t these elements are essential for the maintenance of life. Parey, least as early as 1649, recognized that calcium plays an important t in bodily functions. However it is indeed amazing that so little ntion should have been given to the nutritional significance of gnesium until the present decade. A tremendous volume of litera- e exists concerning calcium and phosphorus. But this, in addition the relatively numerous contributions on magnesium, fails to nish satisfactory answers to some significant problems relating to nutritional roles of these elements. It appears that we are now possession of sufficient knowledge to outline the general facts on ch an intelligent use of calcium, phosphorus, and magnesium is sible in the maintenance of health and the prevention of diseases ted to these elements. A relatively brief discussion of these subjects be the purpose of this chapter.

Content of Calcium, Phosphorus, and Magnesium in the Body.— e calcium, phosphorus, and magnesium content of the body varies r a considerable range depending upon various factors. According Bessey and associates ('35) the skeleton of normally developed adult contains approximately 99.3 per cent of the total calcium in the y. In normal rapidly growing rats and in adult rats on a calcium- diet a somewhat smaller percentage of the total bodily calcium is nd in the skeleton. Even under such conditions, Bessey et al. state the value is of the order of 99 per cent. The skeletal structures tain on an average about 70 to 80 per cent of the total bodily sphorus. But owing to considerable variations in the ratio of etal tissues to soft tissues this value is by no means constant.

About 70 per cent of the total bodily magnesium also is in the s
ton.

With respect to the absolute values for these elements there
marked differences. The average adult human body may con
1400 to 2000 gm. of calcium, or about 2 per cent of the total b
weight. The phosphorus content appears to be even more vari
Approximate figures for the absolute values are 450 to 700
Scarcely anything is known concerning the total magnesium cont

According to Cox and Imboden ('36a) rats at birth contain r
tively small amounts of calcium in comparison to infants at birth.
following table taken from their paper illustrates these differe
and the marked increase in these elements during early growth
the rat.

	Weight	Per Cent Ash	Total Weight		Per Kilo Body Wt.		Per Cent Ash		Ca R A
			Ca	*P*	*Ca*	*P*	*Ca*	*P*	
	gm.		*gm.*	*gm.*	*gm.*	*gm.*			
Rat pup . . .	5.37	1.754	0.0144	0.015	2.68	2.79	15.3	16.0	0.
Human fetus, full term . .	3000	3.33	24.21	13.32	8.07	4.44	24.2	13.3	1.
Rat, 21 days old * . . .	44.7	2.738	0.313	0.236	7.01	5.27	25.7	19.3	1.

* Average of 420 stock animals, 21 days old.

Recalculation of data by Greenberg and Tufts ('36), in terms
this table, shows that normal rats at birth contain about 0.0012 g
of magnesium and at 21 days the value is approximately 0.0151 g
These results indicate that gestation in rats causes relatively less ne
for the minerals in question as compared with the need in hum
gestation.

The forms in which these elements exist in the body are
interest. Since practically all of the calcium is in the bones and te
its principal compounds occur there. Chemical analyses and X-r
diffraction patterns, as well as some other types of evidence, sugg
that it exists in forms such as CaF_2 $[Ca_3(PO_4)_2]_3$ (fluorapatit
$CaCl_2$ $[Ca_3(PO_4)_2]_3$, $Ca(OH)_2$ $[Ca_3(PO_4)_2]_3$, $CaCO_3$ $[Ca_3(PO_4)$
(podolite, and possibly as the mixed apatite), and francol
$CaFCaCO_3$ $[Ca_3(PO_4)_2]_3 \cdot H_2O$. Although no completely satisf
tory solution of the problem has been found it is rather certain th
bone salts are members of the apatite series.

Studies in this field must eventually clarify the relation of ma

ium to calcium in bone. It is the general tendency to dismiss this ment from serious consideration as though it were of no consence. However the ash of normal adult bone contains approximately to 0.8 per cent of magnesium. There is scarcely any information the chemical forms of magnesium in the body. About 20 to 30 per t of the plasma magnesium is non-diffusible. It would be of much erest if compounds of this element should be found in animal ues comparable in any way to that of chlorophyll in plants which tains magnesium as an essential component of the pigment.

The compounds of phosphorus in the body are far more numerous n those of any other mineral element. Some of the known comnds are inorganic phosphate, organic pyrophosphates which inle cocarboxylase (thiamin pyrophosphate), hexose diphosphates, osemonophosphates, glycerophosphates, creatine phosphate, adenylic l (adenine pentose phosphate), inosinic acid, lecithin, kephalin, ingomyelin, nucleoproteins, phosphoproteins, and apatite of bones teeth. Much of the present data on the distribution of phosphorus soft tissues is in terms of such non-specific variables as acidoluble, acid-soluble, inorganic, lipid ester and total phosphorus erman and Quinn, '26; Cole and Koch, '31). This practice, ough superficial, is useful in revealing some aspects of phosphorus abolism. For instance, in typical clinical rickets the serum inorganic sphorus decreases markedly, but scarcely any change occurs in l phosphorus.

Effects of Calcium Deficiency.—Studies on the bodily effects of ium deficiency are necessary to an understanding of the nutritional ificance of this element. One of the first significant experimental stigations of calcium deficiency was made by Aron and Sebauer) whose work is distinctive because they were the first to demonte the advantages of combining chemical and histological proures in the elucidation of a nutritional problem. In this instance problem was rickets but unfortunately no fundamental facts or nts of view were established. Young dogs were given a diet of se meat, maize, and beef fat. After about 2 weeks there was a porary flow of bloody feces. Lacrimation occurred and after several e days the feces were foul-smelling and the appetite failed. rtly thereafter tonic-clonic convulsions intervened. Autopsy showed fication of the bones and a generalized inflammation of the stinal mucosa.

These observations are of considerable interest in relation to the nt report of Martin ('37), who noted that young dogs fed a ified diet containing extremely small amounts of calcium showed ilar degrees of irritability. In this case vitamin D was provided. ddition Martin claimed that total serum calcium was not appreci-

ably decreased. But in view of the resultant tetany it would seem the content of ionizable calcium must have been somewhat red That variable was not determined. Striking effects were the cond of extreme tactile hypersensitiveness, the halisteresis and diffuse of hemorrhage. The latter were observed throughout the bod cluding the viscera, muscles, and bones. Whether it is related failure in ascorbic acid synthesis or metabolism in the dog i known but the appearance of the hemorrhages and the extreme h sensitiveness are suggestive of the scorbutic state. This suggests studies should be made of the possible relationship between cal deficiency and ascorbic acid synthesis.

Rats appear to be more capable of maintaining stability of nervous system in conditions of calcium deficiency. But if vitami is absent the musculature becomes tense. However there is no te (Shelling and Asher, '32; Templin and Steenbock, '33). Templin Steenbock found that adult female rats maintained themselves surprisingly good condition during a period of 8 months during w time the ration contained no vitamin D and only 0.058 per cen calcium. However, loss of weight and evidence of premature sen were observed. The femurs suffered a 10 per cent reduction in whereas the loss was only 6.5 per cent when vitamin D was furnish This, incidentally, is indicative of the value of vitamin D in adultho

Without discussing the question at length it suffices to iten some of the effects of calcium deficiency on young animals as follo

1. Decreased rate or cessation of growth.
2. Probable slight reduction in blood calcium.
3. Changes in intestinal flora.
4. Intestinal inflammation which may or may not be alterna with intestinal atony and inflammation.
5. Hyperplasia of parathyroid glands.
6. Hyperirritability and perhaps tetany.
7. Resorption of bone salts and probably rickets.
8. Negative calcium balance.
9. Premature death.

In adult animals sterility occurs and lactation may be reduced stopped, depending upon the severity of the deficiency. The relat of calcium to lactation is not well clarified but deficiency of t nutrient as the etiological factor in milk fever is supported considerable evidence.

Calcium Deficiency and Milk Fever: Milk fever occurs amo live stock in various parts of the world. A principal symptom is teta convulsions. The disease occurs soon after parturition and the on of lactation, usually in heavy milk producers. A method of treatm widely practiced in former years was inflation of the udder with

mporary respite was afforded by this treatment, apparently owing he arrest of milk production. Studies have shown that the disease haracterized by a reduction in blood calcium. Since various factors, uding calcium and vitamin D-poor diets, and pregnancy, tend to duce a condition of calcium deficiency in the parturient animal, it ot surprising that the sudden demands of heavy milk production uld exceed the animal's ability to maintain an adequate level of od calcium. Proof of the role of calcium in this disease is the matic effectiveness of calcium gluconate, calcium chloride, or some er suitable calcium salt in alleviating the condition. Greig ('31) discussed the subject.

Effects of Phosphorus Deficiency.—Although compounds of sphorus participate in numerous important functions of the body s a significant fact that the literature does not yet contain any orts of experimental studies on the effects of diets very low in sphorus but adequate in other respects. In 1918 Osborne and ndel restricted rats to a relatively satisfactory diet somewhat de- ent in phosphorus but unfortunately their only observations were se regarding growth. Since that time there have been numerous estigations of phosphorus in relation to rickets, but the principal phasis has been on the calcium: phosphorus ratio. A high ratio secured by the addition of a calcium salt, usually calcium bonate, to a basal diet containing considerable amounts of phos- rus. Such diets produce extreme degrees of rickets in suitable mals but they are not a satisfactory means of studying the effects imple phosphorus deficiency.

A difficult problem is the preparation of a suitable diet deficient phosphorus. Almost all sources of protein contain relatively large ounts of phosphorus, and the provision of vitamin B components hout significant amounts of phosphorus has been even more trying. etofore a diet containing 0.13 per cent of phosphorus was regarded pproaching the lower limits of practicability in studies of phos- rus deficiency (Forbes, '37). Day and McCollum ('38) have de- d a diet containing approximately only 0.015 per cent of phosphorus adequate for growth and reproduction when supplemented with sphorus. It consists of: edestin 16.0, gelatin 4.0, sucrose 62.95, salts ree) 4.0, choline hydrochloride in sucrose (1:9) 1.0, cystine in ose (1:19) 1.0, Crisco 10.0, carotene in corn oil (3:1000) 0.3, terol 15 drops per kilo, and vitamin B complex 0.75. The latter is pared from wheat germ and yeast by precipitation of phosphates calcium hydroxide and alcohol. The procedure is too tedious to unt here. Weaned rats restricted to this diet grow slowly for 2-4 ks, then decline and die after 7 to 9 weeks. The effects of the iency are therefore acute and are scarcely without parallel in the

rapidity of death from mineral deficiencies except in the ca magnesium deprivation.

Young rats restricted to the above diet maintain a fairly appetite throughout the first half of the survival period. Marke of bodily calcium occurs but the metabolic balance data indicat some of the phosphorus taken from the bones, with calcium, is ferred to the soft tissues and actually used to promote their gr The animals gradually become inactive and use their legs as as possible. This is understandable since X-ray examination sho progressive rarification which becomes so acute that as death proaches only a faint shadow is formed on the plates, as sh by Follis, Day, and McCollum ('38). Spontaneous fractures occur the lungs and heart become compressed by collapse of the ribs. T appears to be no marked increase in water consumption as is sai occur in phosphorus-deficient animals.

Phosphorus Starvation in Farm Animals: Phosphorus defici in farm animals is reported from many parts of the world (Th and Green, '32). It has been observed in Minnesota, Michigan, P sylvania, Ohio, New York, Virginia, West Virginia, Alabama, Mi sippi, South Carolina, New Zealand, Australia, Hawaii, South Af and various parts of Europe. The deficiency is generally attribut to phosphorus-poor herbage, particularly grass and hay, grown on l phosphorus soils. In several regions the soil depletion has been cau by continuous removal of phosphorus in the form of animals wh were pastured on the land and in others by the continued market of hay and grain. Under natural conditions animals and herb of a given region tend to remain there. The animals die on the la and the fertility is not lost. Mineral deficiencies of pasture lands Australia and elsewhere are now attracting much attention beca the soil has been so depleted by long export of animals as to m the present animals unthrifty and their production unprofitable.

The studies of Eccles, Palmer and associates ('32, '35) have sho that phosphorus deficiency in farm animals is much more wi spread in the United States than was previously supposed. Mine balance experiments with dry and lactating animals showed t a copious milk flow may greatly augment the loss of phosphorus fr the animal's body. It was found that the level of inorganic phospho in blood serum is a useful index to the severity of the disease. T bones become greatly reduced in ash constituents. They have a lo phosphorus and a higher calcium carbonate content than norm bones. Supplementation of the diet with suitable sources of ph phorus, e. g., sodium phosphate, calcium phosphate, etc., causes immediate stimulation of the appetite, more efficient utilization feed, and a return to good health.

An indirect result of phosphorus deficiency in cattle is a disease led *Lamsiekte* (lame-sickness) by South African stock raisers who ve suffered enormous losses from its ravages. The disease is localized d it occurs sporadically. As shown by Theiler in 1919 the disease is intoxication caused by the eating of carcass debris infected with toxigenic saprophyte, *Parabotulinus bovis*. Since cattle suffering m acute degrees of phosphorus deficiency greedily eat all bones d other debris they do not discriminate against carrion even though is reeking with the botulinus saprophyte. In 1922 Theiler and ociates discovered that the craving for bones by such cattle was e to phosphorus starvation. On the provision of any assimilable m of phosphorus such as bone meal, bran, sodium or calcium phos- ate, the perverted appetite, pica, for bones and other debris soon appeared.

Horses and sheep do not develop the habit of bone chewing al- ough in an unthrifty condition from aphosphorosis. Likewise rats tricted to an extremely phosphorus-deficient diet exhibit no symp- ns of pica. Also there is no significant degree of polydipsia in rats. is symptom is said to occur in phosphorus-deficient pigs (Aubel, ghes and Lienhardt, '36).

Effects of Magnesium Deficiency.—Although it has been known many decades that magnesium occurs in considerable amounts in tissues of the body it was not until 1926 that experimental evi- nce was adduced which indicated the indispensability of this ele- nt. Leroy ('26) showed that young mice restricted to a ration taining about 10 parts of magnesium per million failed to grow d died in about 30 days. The nature of the deficiency was not died. McCollum and associates ('31-'35) first described the spectacu- syndrome of magnesium deficiency in rats and dogs.

Young rats 35 to 45 gm. in weight show marked vasodilatation 3 to lays after being restricted to a diet containing about 2 parts of gnesium per million. The condition becomes more pronounced il the 11th and 14th day when it begins to subside. During this iod the animals become progressively apprehensive and show rm at noises or shadows. By the time vasodilatation has begun decrease hypersensitivity may be sufficiently advanced to cause vulsions from disturbances such as the rustling of paper or the sing of air. The onset of a convulsion is striking. The animal es rapidly in a wide circle until it falls on its side. Tonic-clonic vulsions follows. The skin blanches and the eyes protrude. About per cent of the animals die in the first attack. A small per cent l survive several attacks. Those which survive as long as 10 weeks elop an extraordinary fibrous hypertrophy of gum tissue covered a thin layer of oral epithelium. The hypertrophied tissue almost

EFFECT OF MAGNESIUM DEPRIVATION ON THE MINERAL CONTENT OF RAT BONES

INITIAL AGE	FINAL AGE	DAYS ON DIET	MEAN ABSOLUTE VALUES PER ANIMAL FOR FEMORA, TIBIAE AND FIBULAE									
			Dried Bones		Ash		Ca		Mg		P	
			Control	Mg def.	Control	Mg def.	Control	Mg def.	Control	Mg def.	Control	Mg def.
days	*days*		*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
25	25	0	180.5	180.5	79.4	79.4	17.7	17.7	0.5	0.51	17.4	17.4
25	30	5	344.9	285.4	114.1	145.8	35.8	51.1	0.9	0.64	23.7	27.4
25	35	10	256.8	308.1	113.2	156.4	36.1	50.2	0.8	0.66	21.8	32.5
25	40	15	392.6	381.9	184.2	195.9	52.4	66.2	1.5	0.65	40.1	40.7
25	45	20	334.5	375.1	158.8	199.2	52.4	67.7	1.8	0.78	28.9	33.3
25	50	25	362.0	500.5	173.9	276.3	52.5	98.4	1.8	0.86	30.0	47.2
25	55	30*	513.9	591.0	254.8	313.5	87.4	107.8	2.4	0.75	44.6	54.2

* Animals sacrificed in first stage of induced convulsions.

pletely buries the molars. At first this was thought to be due to rption of alveolar bone forming the tooth sockets, but later studies wed that bone resorption does not occur.

Additional effects of magnesium deprivation are cardiac arrhythmia, reased sensitivity to electrical stimuli, marked decrease in serum gnesium, moderate decrease in serum calcium and a large increase erum cholesterol esters. The ultrafilterable fraction of serum mag- ium is lowered in correspondence with the decrease in total gnesium. Consequently the percentage of diffusible magnesium re- ns essentially unchanged. Likewise there is no appreciable change he percentage of diffusible calcium. Calcification of bones is in- sed but the total magnesium content of the skeleton is scarcely to increase at all. This is shown in the table (page 162) adapted n the paper by Orent, Kruse and McCollum ('34).

Several workers, including Cramer ('32), Brookfield ('34), Watch- and McCance ('37), and Greenberg and associates ('38), have nd marked changes in the kidneys. There is not complete unanim- of opinion concerning the exact pathological effects but it appears there is calcification in the cortico-medullary zone, in the pyra- ls, and, at a later stage, in the cortex.

The following table prepared from data published by Tufts and enberg ('38) indicates the effect of magnesium deprivation on magnesium and calcium content of rat viscera. (The results are ressed in mg. of element per 100 gm. of wet tissue.)

CONTENT OF RAT VISCERA

(The results are expressed in mg. of element per 100 gm. of wet tissue.)

ISSUE	CONTROL ANIMALS				MG-DEFICIENT ANIMALS			
	Mg		Ca		Mg		Ca	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
rt . . .	19.3–28.6	22.4	3.6– 7.9	6.4	20.5–26.5	23.1	6.1– 14.7	9.3
cle . .	24.6–35.1	29.6	4.1– 7.9	5.7	15.2–33.5	26.5	7.5– 15.2	10.1
ney . .	16.0–28.0	21.5	6.9–15.4	8.5	15.6–46.7	25.2	14.2– 232	106.7

From the table it appears that the magnesium content of the soft ue is not greatly altered by magnesium deficiency. However the ium content is greatly increased, reaching 15-fold the average value normal kidneys. The diet contained two or more times as much gnesium as the diet used by Orent and associates.

State of Calcium and Magnesium in Foodstuffs and Their logical Availability.—It is understandable that the state of mi in foodstuffs determines in considerable measure their absorptio utilization in the body. The studies of Schmidt and associates (Sch and Greenberg, '35) indicate that much of the calcium and nesium, except magnesium bound in chlorophyll, forms complex with protein in plant tissues. In plant juices these elements a to exist as salts of carbonic, oxalic, citric, malic, tartaric, and such weak acids as occur in plants. They also occur in combin with inositol (hexahydroxyhexahydrobenzene) as the calcium- nesium salt of inositol phosphoric acid. This compound is widely tributed in the plant kingdom, especially in the pericarp of seeds derson, '20), and is generally called phytin.

Calcium and magnesium in milk probably form complex c pounds with proteins but a large percentage may be regarde existing in the form of inorganic phosphates.

The reaction (pH) of the gastrointestinal tract is one of the portant factors in the absorption and utilization of calcium and n nesium as well as phosphorus. Unless the food is made distin acid in the stomach, absorption and utilization are deficient. Thi indicated by numerous types of evidence. Among the more re studies are those of Bussabarger, Freeman and Ivy ('38), who h found that gastrectomy in dogs is followed by marked demineral tion and consequent osteoporosis. Presumably the lack of suffici acid in the initial steps of digestion and absorption was respons for the marked withdrawal of bone minerals.

Factors which tend to keep calcium in solution promote abso tion. Normally the acidified gastric contents upon passing into upper intestine keep the reaction somewhat acid in that port of the alimentary tract. It is believed that this favorable circumsta causes most of the calcium absorption to take place there since lower part of the intestinal tract tends to be alkaline and precipitat of calcium occurs (Courtney, Tisdall and Brown, '28). As shown Zucker and Matzner ('23-24), and others, the feces are alkaline untreated ricketic rats. Administration of vitamin D causes the ret of an acid reaction in the feces simultaneously with clinical impro ment. The relation of vitamin D to the maintenance of proper react (pH) in the intestinal tract is not understood. A critical study of t relationship might be profitable. It is at least interesting that estroge substances, closely related to vitamin D compounds in a chem sense, are capable of quickly producing an acid reaction in the vag which is a normal condition in sexually mature individuals.

In some cases of disturbance in fat metabolism, fatty acids fo insoluble soaps with calcium, thus causing the latter to be excre

t as shown by Boyd, Crum and Lyman ('32) the presence in diet of moderate amounts of fat seems to have a beneficial influce on the absorption of calcium and phosphorus by maintaining a orable acidity of the intestinal contents. The absorption of calcium ps was studied with rats on a fat-low diet. When the calcium ake was 20 to 32.5 mg. per rat per day the utilization values were cium stearate 45 per cent, calcium palmitate 65 per cent, and calm oleate 91 per cent.

The opinion has prevailed for many years that calcium of certain getables is very poorly utilized and that in general the biological ilability of plant calcium is less than that of milk calcium. It quired some time to elucidate the basis for this difference since amin D and some other factors were not controlled in many of the dies. However the best evidence indicates that oxalic acid is the ef factor. Suggestive of this is the correlation of oxalate content of nt foods with the unavailability of calcium. Fincke and Sherman 5) found that calcium of kale was nearly as well utilized as the cium of milk, but calcium of spinach was utilized very poorly if all. That these findings correspond with the oxalate content of le and spinach is indicated by the table of the oxalate content of mmon foods prepared by Kohman ('34). Mackenzie and McCollum 7) fed potassium oxalate to rats and determined the relationship tween oxalate ingestion and calcium retention. At a level of 1.7 r cent potassium oxalate hindered bone growth and calcium retenn when the diet lacked vitamin D but contained 0.35 per cent cium and 0.35 per cent phosphorus. With higher levels of calcium gestion the oxalate was not deleterious. In view of the evidence may be concluded that the liberal consumption of foods containing ge amounts of oxalate might cause an actual calcium deficit unless e diet contains generous amounts of calcium.

Another consideration has been the relative availability of calcium nd phosphorus) from the various kinds of calcium phosphates. Cox d Imboden ('36b) determined the effect of mono-, di-, and tricium phosphates on reproductive ability in rats when the salts were l at the high level of 2.45 per cent. At this level the primary salt s definitely unsuitable and the tertiary form appeared to be slightly perior to the secondary phosphate. Rottensten and Maynard ('34) mpared the assimilation of phosphorus from dicalcium phosphate, P., tricalcium phosphate, C.P., bone dicalcium phosphate, and cooked ne meal. The data seemed to indicate a slight superiority of a secdary phosphate over a tertiary product but it was concluded that really significant differences exist between the four phosphates ted. Presumably the assimilation of calcium from these products similar to that of phosphorus. The data of Higgins and Sheard

('33), who worked with chicks, tend to support the above sions that scarcely any differences in the assimilation exist secondary and tertiary phosphates.

The above discussion has applied almost exclusively to This is due to the almost total lack of critical studies on mag the other element of interest here, and the existence of n data on calcium, which is of much practical importance s ficiencies of it are more likely to occur.

State of Phosphorus in Foodstuffs and its Biological Avai —The ubiquity of phosphorus compounds differing greatly i ical and physiological properties merits considerable attention. ently all compounds of phosphorus in biological material derivatives of orthophosphoric or pyrophosphoric acids. Son compounds of the first are inorganic salts, especially calciu phates of bones and teeth, phospholipids, phosphoprotein, nucleoprotein or its derivatives, hexosephosphates, glycerpho and even flavin phosphoric acid. Pyrophosphoric acid particip the intermediary metabolism of carbohydrate as adenylic aci phosphate. It has been identified also as a component of cocarb (thiamin pyrophosphate).

It became a question of considerable interest at the beginn this century whether the body requires any particular types of phorus compounds to provide for its needs of the various phos complexes. These early studies are reviewed by Forbes and ('14). It was even believed for some time that beriberi was du deficiency of some organic phosphorus complex removed in the ing of rice. Since it is not unlikely that thiamin (vitamin B_1) occur in cereals as a complex with pyrophosphoric acid, that idea based on totally inadequate proof, may have been partly c

An early experiment by McCollum, Halpin and Drescher conclusively proved that phospholipids in general can be readil thesized by the hen since young hens confined to a diet prac free from that class of substances produced many eggs containing more of the complex lipids than the bodies of the birds could p furnish. Investigations of other workers have indicated that ph proteins, nucleoproteins, and various other esters of phosphori can be synthesized by the body. Hence it is generally conclude inorganic sources of orthophosphates can adequately serve t quirements of all species irrespective of age and degree of de ment. In general this is probably true but there is, as yet, n quate experimental proof that it is invariably the case. Future s may show that certain types of organic complexes are utilized efficiently than inorganic compounds of phosphorus. With resp the latter, Shelling ('32) studied the utilization of pyrophos

etaphosphate, and hypophosphite in parathyroidectomized rats and und that pyrophosphate is utilized while the latter two types are ert.

The nutritional significance of phosphoproteins has not received e critical attention this subject deserves. It does not seem probable at casein, for example, quantitatively the most important protein in e diet of all suckling animals, should contain approximately 0.8 per nt phosphorus in ester combination unless the particular complex of nutritional importance and not merely a fortuitous circumnce. As pointed out by Rimington and Kay ('26): "Either this oup (organic phosphorus complex of casein) (a) has some definite rt to play in the economy of the suckling, or (b) is there because certain synthetic necessities of the mammary gland, or (c) is esent adventitiously in the milk, and could be replaced equally well inorganic phosphoric acid as far as the needs either of the mother of the growing suckling are concerned." Schmidt and Greenberg c.), in their extensive review, as well as Rimington and Kay (l.c.), gard the ester-complex as a wise provision of nature, since they ieve the low rate of hydrolytic cleavage (assumed) in the intestinal ct permits most of the calcium to be absorbed before much of the osphate is released. It is assumed that this is a means of preventing formation of calcium phosphate in the gut which they think is apable of being absorbed efficiently. Whether these opinions are id is not known since the subject has not been adequately investied.

It is of interest to consider some of the present evidence concerning mode of combination of phosphorus in casein. Rimington ('27) orted that tryptic digestion of casein quickly releases most of the osphorus as a complex which is relatively resistant to further tryptic drolysis. The complex was called phosphopeptone. Hydroxyglunic acid, hydroxyaminobutyric acid, and serine were isolated after jecting the material to acid hydrolysis. Later Lipmann ('33) reted that serine is the sole phosphoric acid bearing component of casein molecule. This peptide, isolated by mild acid hydrolysis, named serine phosphoric acid. Levene and associates have made ious chemical studies of the phosphoric esters of hydroxyamino ds and in the opinion of Levene and Schormüller ('34) the dipeptide sphoric acid complex of phosphoproteins probably has special siological significance. Since critical nutritional studies have not n reported, no definite conclusions can be drawn. The subject uires careful investigation.

Another subject about which more definite information is at hand e availability of phosphorus in cereals, particularly phytin phosrus. Harris and Bunker ('35) found that the proportion of the

total phosphorus in the form of phytin is variable and may from 30 to 70 per cent in different samples of a single cereal su corn. The phytin phosphorus is poorly utilized. As shown by Mc and Widdowson ('35), in studies on humans, 20 to 60 per c ingested phytin was excreted unchanged. They believed that utilization as did occur was probably the result of bacterial in the intestines. Also it has been shown by Bruce and Callow that the heretofore alleged ricketogenic effect of cereals is due t availability of phytin phosphorus. When oatmeal, for example subjected to mild acid treatment the so-called anticalcifying ten was decidedly reduced. Presumably the slight acid hydrolysis dered more of the phosphorus available to the body. These obs tions were confirmed and amplified by Lowe and Steenbock

In view of these data there is no further basis for Mellanby's p late that cereals contain an anticalcifying substance, designated "toxamin" (American Medical Association, '37). Moreover the no unique advantage to be secured by the irradiation of cerea order to destroy the alleged anticalcifying effect of such foods. principal deduction to be drawn from these investigations is tha chemical value of the phosphorus content of a ration is not necess synonymous with its biological value as respects the available p phorus.

Numerous types of evidence demonstrate the ability of the bod synthesize nucleoprotein, hexose phosphates, and glycerophosph using inorganic phosphates and the appropriate organic substance starting material. In the earlier investigations of rickets consider attention was given to the nutritional role of hexose- and glycerop phates but none of the data suggested significant differences betw the efficiency of their utilization as compared with that of inorg phosphates. However, it has not been conclusively determined whe phosphorus combined in such forms is utilized with any gre efficiency than phosphorus in inorganic forms.

Cations which Interfere with Phosphorus Utilization.—T are various cations which form insoluble compounds with phosph under conditions which exist in the intestinal tract. Some of these iron, aluminum, beryllium, and manganese. Waltner ('27) sho that rickets could be produced in rats by the addition of soluble salts to a non-ricketogenic diet. This was confirmed by Brock Diamond ('34). Severe phosphorus depletion was produced in yo chicks by Deobald and Elvehjem ('35) who treated their nor ration with soluble iron and aluminum salts equivalent to 0.5 and per cent of the theoretical amount required to combine with the die phosphorus as $FePO_4$ and $AlPO_4$. Normal bone ash values of 50 t per cent were reduced to 25 per cent. The latter investigators wa

the clinical usage of very high doses of iron in the treatment of mia might seriously interfere with phosphorus utilization.

A few studies have been made on the effect of beryllium in the duction of rickets (Guyatt, Kay and Branion, '33). When a nor- diet is supplemented with 0.5 per cent or more beryllium carbonate re phosphorus deficiency occurs. This can be prevented by means soluble phosphate administered in sufficient quantity to combine most of the beryllium. Beryllium phosphate appears to have no terious effect.

n view of these data it is safe to generalize that all cations capable orming insoluble phosphate compounds in the intestinal tract will rfere with phosphorus utilization, depending on their concentra- in the diet. Reciprocally, excess of phosphorus in the diet will rfere with the utilization of these cations. The effect on calcium be discussed in relation to the calcium: phosphorus ratio and will be considered in the chapter on iron and copper. Whether utilization of copper and manganese may be seriously affected by lative excess of phosphorus remains to be investigated.

Acid-Base Balance of the Diet and Utilization of Calcium and sphorus.—Various data have been published concerning the rela- ship between acid-base balance and the metabolism of calcium and sphorus. *A priori* it would seem that a preponderance of acids ld augment the excretion of urinary calcium and that an excess lkali would increase the excretion of urinary phosphorus. This ally occurs. But owing to the solubility relationships between cal- and phosphorus, to be discussed in the section on Ca: P ratios, abnormal excretion of one effects an abnormal excretion of the r. However, calcium and phosphorus do not appear to play an ortant role in the regulation of acid-base equilibrium. Goss and nidt ('30) studied the effect of various acid-base levels on the um and phosphorus metabolism of pregnant and lactating rats. basal diets, containing 0.74 to 0.82 per cent of calcium and 0.50 55 per cent of phosphorus, were derived from whole wheat, wheat , alfalfa leaf powder, whole milk powder, casein, butter fat, and d salts. Within the range of acidity employed, no significant rence was found between the diets except that the second litters rats on diets alkalinized with sodium bicarbonate were not nour- by the mothers owing to failure of lactation. Litters continued e diets of their mothers, either alkalinized to the extent of 440 cc. 1 N NaOH per 100 gm. of food, or acidified to the extent of c. of 0.1 N HCl per 100 gm. of food, stored both calcium and horus in the normal manner. These observations support those ub and coworkers who reported that calcium excretion is only ly affected by acid or alkali. And it should be noted that the

diets of Goss and Schmidt contained rather generous amoun calcium and phosphorus. It may be concluded that the dietary base balance, although important in some respects, is relativel slight significance in the utilization of calcium and phosphorus

Vitamin D in the Assimilation of Calcium and Phosphor

The physiological action of vitamin D will be discussed more e sively elsewhere (see chapters on vitamin D) but it is necessary to line here, even at the risk of repetition, some of the data which upon the subject. Calcium and phosphorus assimilation is promote vitamin D but the mechanism involved in this relationship is poorly understood. As pointed out by Bills ('35) this ". . . is ur standable when one contemplates the permutations and combinat which are possible in a system comprising several forms of calc and phosphorus in the blood, a reserve and depository of these ments in bone, a fluctuating intake and output of them in the and excretions, and a dumping place for them in the soft tissues- under the influence of many factors besides the parathyroid gla and the several forms of vitamin D."

Since one of the most apparent effects of vitamin D deficiency i increased loss of calcium and phosphorus in the feces, associated v an elevation of fecal pH, it was early assumed that vitamin D h local action in the gut. But there is practically no experimental s port for this view. For example, it is reported that aqueous soluti of vitamin D favorably influence the extent and density of ossificat in ricketic bone fragments immersed in a solution containing calci (Venar and Todd, '36). Also, the increase in urinary calcium foll ing deprivation of vitamin D is indicative of a systemic rather tha local action.

Another suggested mechanism is that vitamin D promotes calci and phosphorus utilization through its action on the parathyr glands (Taylor, Weld and Sykes, '33). There is considerable lack conclusive proof for this relationship although various experimer procedures have been employed in these studies. It appears signific that young dogs deprived of vitamin D but given satisfactory amou of calcium and phosphorus showed scarcely any change in blo calcium when parathyroid hormone was administered. Control do receiving vitamin D did respond to the hormone (Morgan a Garrison, '33).

The reports of Nicolaysen ('37) are valuable since they indic the nature of critical investigations that are needed. The object of studies has been to determine how vitamin D decreases the fecal a urinary output of calcium and phosphorus. As he points out, all periments up to the present have been performed with considera amounts of calcium and phosphorus in the diet. Hence there is

definite evidence concerning relationships between vitamin D deficiency and absorption or excretion of calcium and phosphorus since the fecal output constitutes both endogenous and exogenous products. The increased output may be due partly to decreased absorption of ingested calcium and phosphorus, partly to decreased secondary absorption of the endogenous moiety secreted into the gastrointestinal tract with digestive fluids, and partly to increased secretion into the gastrointestinal tract. Moreover, it is possible that a deficiency of vitamin D is marked by more or less specific differences in the effect on calcium and phosphorus. Obviously a critical evaluation of calcium- and phosphorus assimilation, as affected by vitamin D, is dependent upon clear-cut information concerning these questions. Unfortunately Nicolaysen's data are not very helpful since his basal calcium- and phosphorus-low diet was not only grossly deficient with respect to several dietary essentials, but it also contained enough egg albumin to produce some toxicity. One of the basal diets, for example, contained agar 4, NaCl 1, egg albumin 20, and sucrose 75. In rats fed this diet the percentage of absorbed calcium, given as the gluconate or carbonate, was considerably higher when vitamin D was fed. The absorption of phosphorus, given as NaH_2PO_4 or disodium glycerophosphate, was complete whether or not vitamin D was administered. This was interpreted by Nicolaysen as evidence that the action of vitamin D in the gut is confined to a direct action on the absorption of calcium. He concluded also that the decreased utilization of phosphorus in vitamin D deficiency, with diets containing calcium, is due to a precipitation by the increased amount of calcium in the intestinal tract. These conclusions are without convincing proof owing to the grossly inadequate diet, but as stated above, the work is significant because it suggests the type of data needed to aid in clarifying the role of vitamin D in calcium and phosphorus utilization.

Parathyroid Glands in the Assimilation of Calcium and Phosphorus.—Lack of space makes it impossible to adequately discuss this subject. Excellent reviews have been written by Thomson and Collip ('32) and by Shelling ('35). It suffices to state only a few of the important facts and opinions. Much experimental evidence suggests that the parathyroids are interrelated with vitamin D in the metabolism of calcium and phosphorus, but without duplication of function. There is no doubt also that a considerable degree of interrelationship exists between the parathyroids and other glands of internal secretion, particularly the thyroid. However, there is very little definite knowledge of the relationships involved.

It is fully established that hyperplasia occurs in the parathyroids when animals are maintained on calcium deficient diets, or in those lacking vitamin D. It is perhaps not without significance that Wilder

and Howell ('36) noted a higher incidence of parathyroid enl ment in the northern part of the United States as compared with sections where the sunshine contains appreciable amounts of violet light. It is suggested, therefore, that deficiency of vitami either directly or indirectly through disturbances in calcium and phorus metabolism, causes parathyroid hyperplasia. A few inves tors have described an enlargement of the parathyroids in chic deprived of vitamin D.

An outstanding effect of parathyroidectomy is the marked crease in serum calcium and the small increase in serum inorg phosphorus. Decalcification of bones occurs. The important wor McLean and associates ('35) has shown that the parathyroids f tion in the maintenance of the calcium ion concentration at a ph logical level. In parathyroidectomized animals the ionized calc may be reduced one-third. Conversely in conditions of extreme hy parathyroidism, induced by parathormone injections, the calcium concentration is almost doubled and the inorganic phosphoru markedly raised (Shelling, Asher and Jackson, '33). These bl changes occur at the expense of the bones. And, in turn, the util tion of calcium and phosphorus is affected, possibly to a certain ex through an interference with renal function.

It is possible to regard the parathyroids as non-essential for since it appears that both animals and man have been maintained an apparently fair state of health for indefinite periods by care regulation of the dietary calcium, phosphorus, and vitamin D. Ho ever, their important role in calcium and phosphorus utilization c not be denied. As pointed out in the discussion of vitamin D the utilization of calcium and phosphorus, the discovery of mea for the preparation of satisfactory experimental diets in which calciu and phosphorus can be varied practically at will should make it p sible to clarify somewhat the role of the parathyroids in calciu and phosphorus metabolism. The basal diet developed by Day a McCollum in the study of acute phosphorus deficiency should be use for the above purpose. By means of such a diet information cou be secured on the relationships between the parathyroids and endog nous and exogenous excretion, as well as of absorption of calcium a phosphorus. Moreover such a diet would permit the regulation at w of magnesium and vitamin D.

Significance of Calcium: Phosphorus Ratio.—It was not recc nized until about 1921 that the ratio of calcium to phosphorus in t diet is of much importance in the promotion of bone growth ai maintenance of good health. Previous to that time most of the empha sis was placed on calcium. Since most of the bone salts consist of son form of calcium phosphate it is remarkable that it took such a lor

ime for the importance of phosphorus to become realized. Early in 921 Sherman and Pappenheimer published an important paper demnstrating that rickets can be produced in rats by a diet containing nly 0.08 per cent phosphorus and about 0.6 per cent calcium. Rickets vas prevented by replacement of some of the calcium, fed as calcium actate, with potassium phosphate. Shortly thereafter McCollum, Shipey, Park and coworkers reported essentially the same results. Thus hese two papers marked a distinct advance in the subject of mineral utrition since they indicated not only the necessity of adequate phosphorus in rickets prevention but the importance of the dietary calcium: phosphorus ratio.

Upon consideration of the physico-chemical relationships between alcium and phosphorus it is understandable that a relative imbalance in the dietary content of these nutrients should render impossible their proper utilization. Practically all of the calcium in the body is deposited in the skeleton as some form of calcium phosphate, the solubility of which at the reaction (pH) of body tissues and fluids is very sparing. Since the bodily fluids, containing dissolved and ionized calcium and phosphorus, are contiguous with the solid calcium phosphate of bone, it follows, on the basis of mass action relationships, that a reversible equilibrium must exist between the two phases. Moreover, there is a continuous interchange of bodily fluid components, including calcium and phosphorus, with the gastrointestinal contents. Also there is a continuous although variable excretion of these nutrients in the urine. It is therefore apparent that if the dietary calcium-phosphorus ratio is very abnormal, as respects the ratio in the body, both elements will be excreted and the bodily calcium and phosphorus will tend to be withdrawn. An indication of this effect is afforded by the work of Shohl and Wolbach ('36). The following table prepared from their data is sufficient to illustrate the point:

Composition of Diet			Bone Ash	Blood Serum	
Ca : P	Ca	P		Ca	P
	per cent	*per cent*	*per cent*	*mg. per cent*	*mg. per cent*
1	.25	.25	46	6.0	11.4
1	.50	.50	48	7.6	10.0
1 : 8	.25	2.00	40	6.3	15.2
1 : 4	.50	2.00	42		11.2

It will be noted that a low ratio, in spite of the adequacy of calcium in absolute amounts, causes poor mineralization of the bones. An

abnormally high ratio, not shown here, causes even more delet effects. This is shown by the studies of Kletzien, Templin, Stee and Thomas ('32) who found that adult rats lost calcium and phorus from their skeletons when kept on the Steenbock and ricketogenic diet which contains about 1.25 per cent calcium about 0.25 per cent phosphorus. This loss was reduced but not vented by the addition of vitamin D.

This introduces the question of optimum calcium: phosph ratios. As pointed out by Sherman ('37), an optimum ratio pro should be defined in terms of the particular nutritive requiren since these may be variable. A suggestion of this is furnished by studies of Stearns ('31) who considers that a retention ratio of calc phosphorus between 5:1 and 2:1 is normal for an infant, but older children the ratio is somewhat lower, about 1:1, owing to relatively greater amount of phosphorus required for soft ti growth. Indirectly this suggests the optimum dietary ratios of calc and phosphorus for infants and children provided these nutrients furnished in a utilizable state.

Cox and Imboden ('36), using a purified diet, concluded th calcium: phosphorus ratio of 1.0, at a calcium level of 0.49 per c is the ideal mineral level and ratio for successful gestation and la tion in rats. They found also that phosphorus in excess was be tolerated than calcium in excess. The experimental basis of their w was sound but attention must be called to the fact that no source vitamin D was provided.

Possibly discussions of rickets have tended to stress calcium: ph phorus ratios to the extent that the necessity of maintaining adequ absolute levels of these minerals in the diet has been minimiz Rickets does occur in rats when the calcium and phosphorus cont of the diet is quite low, but the ratio is approximately norm Shohl and Wolbach (l.c.) observed rickets when the ratio 1:1 but unfortunately the experimental diet was composed larg of cereals. Hence the exact amount of available phosphorus was known.

If there are different optimum ratios for various stages and fu tions of life it is not probable that the differences vary significan in a practical sense, from the generally accepted range of 1 to 2. view of the recent studies which show that phosphorus in cereal only partially available, it is necessary to be cautious in accept data and conclusions based on the use of cereal diets. Unfortunat most of the present information is of that sort. The high-cereal rick producing diets of McCollum and of Steenbock and Black served extremely useful purpose when the fundamental aspects of rick were being established but it appears that the peak of their usefuln

research has passed. Profitable work of the future must be based the use of purified diets.

Role of Magnesium in the Metabolism of Calcium and Phosrus.—The evidence is predominant that large amounts of magium in the diet is a disturbing factor especially in the utilization of cium. The view that there is a physiological antagonism between ium and magnesium originated with Loew during the latter part he last century in studies of plant nutrition. In 1905 Malcolm fed gnesium chloride to dogs and noted a small increase in calcium retion. Eight years later Hart and Steenbock found that magnesium oride and magnesium sulfate fed to swine caused an increased retion of calcium in the urine but not in the feces. This observation made really significant by the further observation that soluble sphates fed with the magnesium caused a decrease in the loss of nary calcium with scarcely any increase in the fecal calcium. ther studies by Palmer, Eckles and Schutte ('28) with cattle sisting on phosphorus-low diets confirmed the favorable effect of sphates in the sparing of calcium when the diet contains large ounts of magnesium.

Cunningham ('33), working with rats, observed a reduction in e calcium and an increase in magnesium when magnesium was in the form of sulfate or carbonate. This did not occur when gnesium phosphate was fed. Moreover, Buckner and associates) found that magnesium, as the carbonate, disturbed the mineral abolism of chickens. The basal ration consisted of yellow corn 80, at middlings 20, NaCl 1, and cod liver oil 2, with skim milk ad um. Different groups of 30 day-old chicks received supplements of $(PO_4)_2$; $CaCO_3$; and $MgCO_3$ in various proportions. Feeding of CO_3 decreased the percentage of Ca and increased the percentage and Mg in the leg bones.

tudies on rats by Elmslie and Steenbock ('29) contradicted the relaship between calcium, phosphorus, and magnesium, as indicated . The explanation of these divergent findings is not apparent, but e can be scarcely any doubt that magnesium does interfere with um metabolism, especially in conditions of inadequate phosus intake. There is no conclusive explanation of this relationship, d on experimental evidence, but it appears possible that the excess nesium is excreted, in part at least, as phosphate, thus rendering e of the calcium unassimilable and resulting in its excretion.

ndirect evidence that magnesium affects calcium utilization is ded by the numerous observations on physiological antagonism een these minerals, referred to above. Classic experiments on subject are those of Meltzer and Auer (1908) who found that anesthesia could be induced by the injection of magnesium salts

and that the effect could be prevented by means of calcium salts. relationship has been thoroughly established by numerous e ments.

The recent studies of magnesium deficiency in rats and dog bear on the subject. Day, Kruse and McCollum ('35) found that restricted to a diet deficient in both magnesium and calcium longer than similar animals deprived only of magnesium. Mor most of the animals failed to exhibit symptoms of hyperirritabil principal feature of the magnesium deficiency syndrome. Also, and Greenberg (l.c.) have shown that a high content of cal in the diet (1.16%) increases the severity of magnesium defic and raises the amount necessary to meet the minimal magne requirements.

Calcium, Magnesium, and Phosphorus in Tetany.—A discu of calcium, magnesium, and phosporus in nutrition is not ade without giving some consideration to tetany. Hess ('29) has wr an admirable account of this condition in which he states that te ". . . is merely a symptom-complex resulting from and giving dence of, an increased irritability of the nervous system and ma incited by a variety of factors." In general the various forms of te are characterized by one or the other of two conditions, namel reduction in blood calcium or a lack of change in blood calc Nearly all cases of tetany seen clinically are of the low-calcium but, as shown by Kruse, Orent and McCollum, magnesium deficie is characterized by tetany without apparent involvement of calc or phosphorus. On the basis of numerous studies of irritability it become established that the proper irritability of a tissue or organ depends upon the ionic balance of all cations and anions in the circu ing fluids. At present calcium and magnesium may be regarde the most important in this respect.

According to the significant studies of McLean and others, 50 t per cent of the total serum calcium is ionized, the remainder be combined with protein. About 65 to 80 per cent of the total se magnesium and apparently all of the inorganic phosphorus are ioni

In absolute terms normal adult human blood serum contains ab 10 mg. of calcium, approximately 2.0 to 3.0 mg. of magnesium, about 3.5 to 5 mg. of inorganic phosphorus per 100 cc. In grow children the content of inorganic phosphorus is about 4.5 to 6.5 per 100 cc. but there is no appreciable difference with respect to cium and magnesium. All of the unionized calcium appears to bound to protein and presumably this is the state of unionized m nesium. As shown by McLean and Hastings ('35a) the total calci total protein, calcium ions, and bound calcium are all interrelated one equilibrium, which as a first approximation can be represe

a simple mass-law equation yielding the ionization constant of ium proteinate. But as they have further pointed out (McLean Hastings, '35b), this equilibrium is affected by such variables as temperature, albumin to globulin ratio (for want of a better term xpress differences in the quality of serum protein), and mag- um. Since hyperirritability and tetany intervene when the concen- on of ionized calcium is even slightly reduced from the physio- al range, about 4.25 to 5.25 mg. per 100 cc. of serum, it is ap- nt that a well-regulated mechanism must exist to maintain con- y as respects this factor. As implied previously, the parathyroid ds play the most prominent role in this function. Parathy- ectomy causes a reduction of ionized calcium and parathormone eases the level above normal.

But the regulatory action of the parathyroids is dependent upon calcium and phosphorus content of the diet, as well as the vitamin take. When the vitamin D intake is inadequate calcium-low diets ys cause a decrease in total serum calcium, and presumably a nution in the ionized moiety since hyperirritability intervenes. same effect is caused by adding considerable amounts of phos- us to a diet containing calcium. Presumably this is explainable he basis of calcium:phosphorus ratios, already discussed. The roles cid-base equilibria, serum protein, and other factors are of less ical significance and are afield from the present problem.

The role of magnesium in tetany has not been clarified although is scarcely any doubt that a deficiency of this element causes a ue tetanic syndrome. As reported by Hoobler, Kruse and Mc- m ('37) the diffusible (ionized) calcium of magnesium-deficient although reduced, did not reach the critical tetanic range. The tion in diffusible magnesium was marked. Hence it was con- d that ". . . the tetanic syndrome of magnesium deficiency was y associated with the extremely low concentration of magnesium in the blood."

is significant that Hirschfelder ('34) observed low serum mag- m values (0.9 to 1.37 mg. per cent) in all of a series of 10 patients festing muscular twitchings or convulsions. The cases were diag- as follows: 1 parathyroid tetany, 2 epilepsy, 1 cerebral injury, thers acute or chronic nephritis. Three of the latter were given esium sulfate orally. This was followed by a rise of serum mag- m to 3.3-5.8 mg. per cent and a disappearance of the nervous toms.

Grass Tetany" in Cattle: Although Hirschfelder's observations practically alone as respects indications of hyperirritability from esium deficiency in humans, there is a form of tetany in cattle n in Holland as "grass tetany," which is sufficiently extensive

to constitute an economic problem. In some parts of Holland lac cows taken from stalls where they have been on dry feed and on spring pasture, following calving, have characteristic tetanic se which may terminate in death. Following Orent, Kruse, and Collum's description of tetany in dogs and rats deprived of magne Sjollema and coworkers ('32) noted the similarity between that drome and the grass tetany with which they were familiar. An of blood from cows in this condition showed the following in ter mg. per 100 cc. of serum: calcium 6.65, inorganic phosphorus 4.33 magnesium 1.66. The phosphorus was approximately normal bu values for calcium and magnesium were only about two-third normal. In his early studies Sjollema found that intravenous i tions of calcium chloride solution caused an immediate curative e In later studies he reported the ineffectiveness of calcium gluco which is so effective in milk fever, and emphasized the importanc restoring a normal level of blood magnesium in order to cure or vent the disease. Apparently there is a deficiency of both calcium magnesium in this syndrome, produced by inadequate diet and heavy drain on reserves occasioned by heavy milk flow. Howeve deficiency of magnesium appears to predominate. As shown by I Kruse and McCollum (l.c.) tetany is not likely to occur in c deprived of both calcium and magnesium. Apparently the nor calcium: magnesium ratio of the blood must be altered before hy irritability can occur.

Further indication that magnesium tetany may occur under cert dietary conditions is provided by the interesting observations of Dun and associates ('35). Calves reared on a diet of whole milk sup mented with iron, copper, and manganese had normal serum calci and inorganic phosphorus values but the level of magnesium considerably reduced. A tetanic syndrome was observed which sembled that occurring in rats and dogs deprived of magnesium. Ad tion of magnesium salts to the diet prevented or cured the syndro This suggests that the cow's milk either contained insufficient amou of magnesium to promote normal growth or, under the conditi of the experiment, magnesium was poorly utilized. Day and Ore Keiles ('38) studied young rats restricted to a whole cows' milk c supplemented with iron, copper, and manganese. They were unable note hyperirritability or abnormal values in serum magnesium, cium, and inorganic phosphorus.

Calcium, Magnesium, and Phosphorus in Ossification.—Me anisms of ossification are only vaguely understood. Two basic conce of the process are: (a) the idea of supersaturation of blood ser with calcium and phosphate ions and a precipitation of these as bo salts in cartilage when local conditions are favorable; (b) the vi

bone salts are formed through the action of an enzyme (phos-tase) in cartilage which hydrolyzes organic esters of phosphoric producing thereby a local concentration of inorganic phosphate and resulting in the precipitation of calcium phosphate of the position characteristic of bone.

important objections can be raised against the supersaturation ory. One defect is the lack of evidence that the mineral structure one bears any resemblance to the critical ion product postulated supersaturated blood serum. The theory was advanced by Holt, Ier and Chown ('25) who were led to believe on the basis of their eriments, that blood serum is normally supersaturated with ter- calcium phosphate to the extent of more than 200 per cent, for shaking serum with solid calcium phosphate there is deposition of salt, while the content of calcium and phosphate decreases in ivalent amounts. No such deposition occurs when serum alone aken without the addition of the solid phase. They suggested that serum bathing the bone matrix readily gives up its surplus, causing ification. As pointed out by Stewart and Percival ('28), Holt and ciates underestimated the complexity of the ionic equilibrium and ored the fact that about 50 per cent of the blood calcium is in com-tion with protein.

The view that organic esters of phosphoric acid play the leading in bone formation merits consideration since it is the most plausible he present theories. This concept was advanced by Robison ('32) le studying the hydrolysis of hexosemonophosphate by various ymes. As he states, "For these experiments the readily soluble ium and barium salts were used, and when hydrolysis occurred liberated inorganic phosphate was precipitated as the insoluble ium or barium phosphate on the sides of the vessel." This observa- suggested to Robison the possibility that such a mechanism might oncerned with the deposition of calcium salts in bone. Investigation ved that bone does contain a very active enzyme which can hydro- not only hexosephosphates but also other phosphoric acid esters. ison's theory is made more plausible by the finding that the enzyme sent from cartilage in which ossification is not taking place. It was vn that calcification occurs in slices of young bone, whether from mal or ricketic rats, when these are placed in a solution of calcium osephosphate at the correct pH.

Shipley and coworkers ('24, '26) found that no calcification occurs n the cartilage and bone of ricketic rats are incubated in the serum icketic rats. On the other hand they found that calcification does ur when a piece of the cartilage and bone from the same ricketic mal is incubated in the serum of normal rats. Since the inorganic sphate value of the ricketic serum was only 2.5 to 3 mg. per

100 cc., as contrasted with the normal value of 8 mg., it was sugge that *in vitro* calcification is somewhat similar to that *in vivo*. T further studied the calcification of bone and cartilage slices immerse sterile solutions of inorganic salts containing calcium and inorg phosphorus in concentrations similar to those in plasma. It is signifi that the presence of only 1 or 2 per cent of egg albumin inhibited cification, although the serum contained 10 mg. of calcium and 4 of inorganic phosphorus per 100 cc. In the absence of protein calcif tion readily occurred. This effect of protein was not explained Shipley and associates concluded that in rickets failure of calcificat is not caused by any primary inability of ricketic bone or cartilage undergo calcification, but that it is attributable to an insufficient c centration of the necessary elements in the fluids bathing the bone sues, which must exceed a certain minimum value.

Robison, however, pointed out that the above solutions, in t absence of protein, were highly supersaturated with respect to norm plasma. This is evident since not more than 5 or 5.5 mg. of calciu in normal plasma is ionized, the remainder being bound by prote

The evidence in favor of the phosphatase theory is indeed impr sive. For instance, Robison and associates have shown that *in vit* calcification can be made to take place with relatively low levels calcium and inorganic phosphorus if a phosphoric acid ester is adde in very small amounts. As little as 0.5 mg. of phosphorus in this for is sufficient. Since the blood normally contains no more than 0.5 m per cent of phosphorus as phosphoric acid esters it has been suggeste that phosphatase action cannot be at the basis of calcification, owin to the low concentration of its substrate. However, it would seem tha an equilibrium might exist between inorganic phosphorus and th esterified forms entering into the calcification process. By means o such a mechanism the concentration of phosphoric acid esters coul be maintained at a constant physiologic level and calcification coul proceed at an unvarying rate. Experiments should be made to deter mine whether such an equilibrium occurs. On the whole, Robison' views seem to explain the process of calcification better than any othe The fact that phosphatase occurs in kidney, intestine, blood, and othe tissues appears to be a disconcerting fact difficult to reconcile wit Robison's theory. It is probable that there is more than one phos phatase, as discussed by Kay ('32), Robison ('36) and Linderstrøm Lang ('37).

Mobility of Calcium, Magnesium, and Phosphorus in Bones an Teeth.—A great deal of attention has been given to the study o calcification as compared to the interest in mobility of calcium, mag nesium, and phosphorus after they have entered the skeletal structures It appears self-evident that an understanding on the mechanism o

mineralization" of bone and teeth, if such occurs in the latter, and bility of skeletal minerals are important.

As shown by Aub and associates (Bauer, Aub and Albright, '29), other workers, much of the calcium and phosphorus deposited in es may be regarded simply as a reserve supply to be used in time of d. Presumably some of the magnesium in bones may be regarded as illing a similar function. This storage capacity of bones is a function he trabeculae. The content of minerals in the cortex is not appre- ly influenced except in the case of very unusual bodily demands.

Recently it has become possible to determine more precisely the rement of phosphorus in the body by means of radioactive phos- rus. It may be presumed that this provides some indirect means stimating the mobility of calcium, since practically all of the calcium ombined with phosphorus. The radioactive isotope of phosphorus a half-life value of 15 days, making it possible to utilize this sub- ce as an "indicator" in metabolic investigations. The concentration adioactive phosphorus can be readily estimated by means of a ger counter. Also, there is little reason to believe that the small unts of material required for a metabolic experiment would have unnatural effects as to invalidate the data.

Chiewitz and Hevesy ('35) fed rats a few milligrams of sodium sphate containing radioactive phosphorus as indicator. By means issue analysis and mineral balance studies it was estimated that average time a phosphorus atom spends in the body of a normal s about 2 months. They found that in the adult rat about 30 per of the phosphorus atoms deposited in the skeleton were removed ne course of 20 days. Since calcification, in the sense of bone growth, practically ceased in mature rats it must be concluded that the osited minerals are not "fixed" but continually in the process of ilization, either from one part of the body to another, or excreted replaced by the dietary source and the "moving" phosphorus in body. Even the molar teeth, which do not grow in adult rats, took ome of the radioactive phosphorus. Hence it may be inferred that mineral content of teeth is not completely fixed. This has important ications as respects relationships between the quality of diet and al caries.

Pathological Calcification.—Owing to the relative insolubility of e calcium salts at the pH of bodily tissues and fluids, abnormal fication is a problem of some importance from the viewpoint of ition, since dietary procedures can be used in its control. The ques- has been extensively discussed by Barr ('32). In general it may tated that deposits of calcium salt tend to form in any dead or g tissue and any nutritional factors which lead to devitalization of sue will favor calcification. A significant example is the formation

of renal calculi in conditions of vitamin A deficiency (Higgins, The epithelial tissue of the urinary system undergoes marked cha (see chapters on vitamin A) and owing to the secondary invasio microorganisms ammonia is formed by fermentation of urea. induced local alkalinity causes the precipitation of calcium phosph These masses of insoluble material become large enough to obst tubules and ducts, thus causing serious damage.

As shown by Cox and Imboden ('38a, l.c.) and others, high calci diets in the absence of vitamin D also cause the formation of re concretions consisting of calcium phosphate. Another example is fr the work of King (Chapter XVII) who showed that histologi changes suggestive of arteriosclerosis occur in the blood vessels guinea pigs whose reserves of ascorbic acid are low. The lesions caused by poisons of bacterial origin since there is not sufficie ascorbic acid to inhibit bacterial action. It is probable that blood vess injured by such agencies will calcify after some time. Conditions fav able to calcification may be brought about by various factors such temporary interruption of circulation, and poisoning from mercu iodine, iodoform, and other substances.

The Calcium Requirement of Man.—Since absorption and rete tion of calcium and phosphorus is dependent upon rate of growt ionic relationships between these elements and others which for compounds of low solubility, the supply of vitamin D, and even th composition of the diet as respects other nutrients, it is not surprisin that experimental data on the calcium and phosphorus requirement infants, children, and adults, although available from numerous studie do not lend themselves to simple presentation or unequivocal inte pretation. Leitch ('37) has prepared an excellent review of the subjec As is true of other nutritional factors it is difficult to determine the be criteria on which judgment should be based concerning requirement for calcium. Certainly the maintenance of a positive calcium balanc even in adults, on a given dietary regimen is not entirely a valid basi for judgment. In this case, for example, it is possible that a slight bu continuous positive balance is no more indicative of an optimal nutr tional state, as respects calcium, than is true of positive nitrogen ba ances in determining the requirement for protein.

A great many mineral balance studies of humans and animals hav been made as a means of estimating the calcium needs. Conclusion based on such procedures are subject to considerable error since the are usually too short to constitute an adequate sampling of the minera exchange. Macy and associates ('36), whose contributions based o mineral balance procedures are well known, have a realistic attitude i this matter. They have observed in children marked fluctuations in th balance of various minerals and nitrogen, although the subjects wer

parent good health. A short interval of sampling in such subjects, h are presumably typical, would give quite erroneous information. mphasized by Leitch, the only adequate general criterion is the t of various dietary regimens on the health of man observed over periods of time.

he body of the newborn infant contains approximately 7 to 8 gm. lcium per kilogram (birth weights averages 3 kg.). During the 6 to 12 months the value is increased to about 12 to 15 gm. per ram. At 2 to 3 years of age it is 20 to 24 gm. per kilogram. The rises steadily (on the basis of statistical analysis) until at 9 to 10 of age the content is about 35 to 36 gm. per kilogram, after which atio of calcium to weight remains essentially constant up to at 18 years of age. But it should be recognized that these data are on a paucity of reliable information. On the basis of standard ge growth records it may be estimated that the daily requirement *ained calcium* at 6 months is about 0.3 gm. and rises as the child s. Daniels and associates ('35) have concluded that normal chil- between 4 and 7 years of age require 45 to 50 mg. of calcium in liet per kilogram of body weight. As shown by Hubbell and ne ('34) girls between 7 and 11 years of age retained about 5 mg. cium per day per kilogram of body weight. Stearns ('31, l.c.) be- that average daily retentions of calcium (and phosphorus) of nan 10 mg. per kg. of body weight are probably not optimal in young children. Leitch has summarized his views regarding the minimum calcium requirements, assuming that 50 per cent of mount ingested is absorbed. They are: from 6 months to 2 years, n.; from 2 to 9 years, 0.9 gm.; 1 gm. after 9 years and increasing gm. between 15 and 16 years. He assumes that thereafter the nt may be gradually decreased to the adult maintenance require- This value is estimated as 0.55 gm. per day.

fants commonly absorb both calcium and phosphorus rather y, even from breast milk. Leitch concluded that it is impossible e breast fed infant to maintain birth composition of the skeleton g the first 6 months since the average content of calcium in n milk is only 0.32 gm. per liter. In comparison, cow's milk con- about 1.20 gm. of calcium per liter. But when all factors are con- d human milk may be regarded as superior to cow's milk. There ne reason to believe that normal infants at birth have a store of m in the bone trabeculae which is drawn upon during the first nonths of postnatal life (Bauer, et al., '29, l.c.).

ne classic studies of Sherman and Hawley ('22) are generally d concerning the calcium requirements of children. On the basis ance studies they concluded that children from 3 to 13 years e require an intake of 1 gm. of calcium per day if most of

this nutrient is furnished by milk. It requires about 1 quart of m to furnish 1 gm. of calcium. As pointed out previously the calc of some foods, especially certain vegetables, is poorly utilized bec of their oxalate content.

Owing to the rapid skeletal growth which normally occurs adolescence it must be assumed, for want of absolute evidence, the calcium (and phosphorus) needs are particularly large. To ('34) extensive roentgenological data indicates that skeletal rarifica is a common occurrence in this period. Hence there may be con erable basis in Leitch's belief that 1 gm. of calcium per day is optimal. But actually we have no real evidence in support of tacit assumption that the skeletal structures of children and grow animals should be kept as fully saturated as diet can make th Since it is definitely known that a large percentage of children not receive enough calcium, this view should not be misinterpret As a general policy intelligent attempts should be made to incre the consumption of calcium-rich foods.

The unincumbered adult, according to Sherman's estimatio should have a minimum of 0.4 gm. of calcium per day. It is gen ally believed that this value should be increased by 50 per cent order to assure an adequate intake. Hence the normal value may regarded as about 0.68 gm. per day. That amount of calcium furnished by a little over 1 pint of milk. Owing to the tendency hypochlorhydria in adults past middle life it is probable that th calcium absorption is considerably diminished. Osteoporosis of var ing degree is common in older people. This condition is probab brought on by long periods of insufficient calcium intake combin with failing calcium absorption. Since there is a lack of critical studi on calcium (and phosphorus) metabolism in persons who are pa middle age, generalizations regarding the requirements cannot made.

The requirements in pregnancy and lactation are obviously large According to studies by Coons and associates ('35) there is a hig storage of calcium, as well as iron and nitrogen, in early human pre nancy which is in apparent excess of fetal needs if the dietary co ditions are favorable for storage.

Coons and Hunscher have estimated that pregnant women sto about 200 mg. of calcium daily from the 4th to the 9th month pregnancy and 300 mg. daily during the last week of pregnanc Macy and associates ('30, '31) have made extensive investigation They found that women producing large amounts of milk were fr quently in negative calcium balance although the diet, of high quali in every known respect, contained 3 to 4 gm. daily of calcium an phosphorus. Calcium and phosphorus in the diet of lactating wome

utilized more economically when cod liver oil and yeast were inistered.

here are no satisfactory absolute figures for calcium requirement ng pregnancy and lactation. On the basis of such evidence as we it would seem that the diet should furnish 1.5-3.0 gm. of calcium day during pregnancy and that this value should be increased, aps to 2.0-4.0 gm. during the lactation period. This amount is rge that it cannot be provided by any common dietary constitu- except milk. Either tertiary or secondary calcium phosphates are actory supplements to the diet of pregnant or lactating women. t is advisable to rely upon milk and other suitable natural foods uch as possible to furnish calcium.

he Phosphorus Requirements of Man.—In general the utilization osphorus is influenced by the same factors discussed in the sec- on calcium. Also, the criteria upon which phosphorus needs are ated differ in no significant way from those used in estimating um needs. Quantitatively, however, there are differences. Phos- us is required for numerous purposes in addition to the formation nes and teeth. Nearly all of the bodily calcium contained in the ton is combined with phosphorus. But large amounts of phos- us occurs in the soft tissues unassociated with calcium. On this Stearns ('31, l.c.) has concluded that a Ca: P retention of less 1.5: 1.0 is indicative of rapid muscle or tissue growth, and a tion ratio of approximately 2 suggests that nearly all of the phos- us is being deposited in the bone. Perhaps owing to the likeli- of calcium deficiency in persons who do not regularly drink milk e milk products which contain calcium, much more attention been given to the estimation of calcium requirements than of phorus requirements. Many common foods contain considerable nts of phosphorus; hence it is somewhat tacitly assumed that hosphorus need tends to take care of itself. Moreover, unless m is ingested in considerable amounts as the carbonate or nate, for example, it is very unlikely that the phosphorus intake ver lag behind that of calcium. Milk, the principal source of m, particularly of infants and children, contains almost 1 gm. osphorus per quart; the calcium: phosphorus ratio of milk is 1.2: 1. The most desirable dietary Ca: P ratio, as discussed pre- y, is about 1: 1.

cording to Daniels and associates the phosphorus needs of chil- 4 to 7 years of age is about 60 to 70 mg. per kilogram of body- t. Hence a child weighing 20 kilograms should receive 1.2 to n. of phosphorus per day. As a general rule it is regarded that osphorus requirements are about 25 per cent higher than those lcium. Sherman calculates that a normal adult should receive

at least 0.88 gm. of phosphorus daily. The values for pregnant lactating women may be regarded as about 25 per cent higher those for calcium.

The likelihood of phosphorus (and calcium) deficiency in nancy, unless the diet contains very generous amounts of this nu is indicated by Cox and Imboden ('36c, l.c.) who compiled data the studies of Toverud (1931). The table is as follows:

Mineral	Level of Intake	Average Intake	Number of Metabolism Periods	Average Month of Pregnancy	Neg. Bal. Per
		gm.			
Calcium .	less than 1 gm.	0.83	17	7.6	41
Calcium .	more than 1 gm.	1.35	27	7.6	14
Phosphorus	less than 1 gm.	0.82	6	7.0	83
Phosphorus	more than 1 gm.	1.48	38	7.7	13

The Magnesium Requirements of Man.—Scarcely anythin known concerning the human requirements for magnesium. Simi little is known about animal needs for this nutrient. It has pointed out that calves restricted to a whole milk diet for periods of time apparently suffer from lack of sufficient magnes Denis and Talbot ('21) noted low plasma magnesium values normal calcium levels in certain children with various types of eases. Hirschfelder's observations of low plasma magnesium valu some human cases of hyperirritability have been discussed. Thus t are indications that some degree of magnesium deficiency might o in humans.

Daniels and Everson ('36) attempted to estimate the magnes needs of children 4 to 7 years of age. Since the output of m nesium in urine tended to parallel the amount ingested, they ado this relationship as a criterion of magnesium need. For example, values for urinary magnesium, when coexistent with low retenti were regarded as indicative of too low ingestions. High urinary m nesiums with high retentions following high ingestions were beli to indicate that the subject had been receiving previously less t the optimum amount. It was found that 75 per cent of the chil studied were in the latter group. On this basis it was tentatively cluded that the diets of children should contain not less than 13 of magnesium daily per kilogram of body weight.

Nothing is known concerning the magnesium requirements unincumbered adults and pregnant or lactating women.

Factors Affecting the Content of Calcium, Magnesium, and Phosphorus in Foodstuffs.—The composition of soils and the vagaries weather are important factors in determining the mineral composition of plants and vegetables. Since man, and most of the animals on which he depends for foods, are directly affected by the amount calcium, magnesium, and phosphorus in products of the soil, the ent subject is one of obvious moment. It has been indicated that e areas of land contain such low amounts of phosphorus that anis indigenous to those areas are scarcely able to live. The data of t, Guilbert and Goss ('32) show that the phosphorus content different grasses may vary from 0.03 to 0.68 per cent. Calcium vs similar wide variations, ranging from 0.10 to 2.90 per cent. op's ('34) analysis of Alabama vegetables grown on different types oil showed that the calcium and phosphorus content of a given table varies widely, usually in opposite directions, so that extremes he Ca: P ratio may result. Moreover, it seems probable that these avorable ratios result in excessive excretion of magnesium, thus ling to cause a magnesium deficiency. This view is supported by following data supplied by Forbes and Keith (l.c.) showing the position of certain American foodstuffs:

CIUM, MAGNESIUM, AND PHOSPHORUS PER 100 PARTS OF DRY SUBSTANCE

	Ca	Mg	P
	gm.	*gm.*	*gm.*
beans	0.230	0.224	0.649
ed oilmeal	0.403	0.544	0.786
uts	0.068	0.180	0.399
(maize)	0.012	0.108	0.260
	0.102	0.118	0.395
(skimmed)	1.336	0.146	0.979
rass	0.336	0.240	0.242
r hay	1.236	0.292	0.183
a hay	1.130	0.400	0.238
t hay	0.326	0.262	0.173

ccording to Sjollema the winter rations of cows which develop tetany include hay, a large per cent of maize and other cereal s, and concentrates such as extracted soybean meal and peanut It seems probable, therefore, that depletion of magnesium in grass y is due to deficient retention rather than to an insufficient supply s element.

so, on a given type of soil one strain of a given plant may have iderably different content of a given nutrient than another plant

grown under similar conditions. This is illustrated by the studie Greaves and Greaves ('33) working with wheat. Two varieties grown on the same soil under similar conditions. The following t which includes values for protein, potassium, iron, and sulfur, i cates the pertinent results:

PERCENTAGE COMPOSITION

VARIETY	PROTEIN	Ca	Mg	P	K	Fe	
Kota	17.41	0.080	0.182	0.308	0.334	0.006	0.
Turkey	14.42	0.055	0.159	0.153	0.276	0.006	0.

Obviously one is not justified in assuming that the composition a given ration, made up of natural foodstuffs, can be reliably estima on the basis of classic food analysis tables. Proof of this is indica by the fact that the skeletal development was superior in rats fe diet containing 90 per cent of Kota wheat, as contrasted with th fed a similar basal ration containing 90 per cent of Turkey wheat.

Milk, eggs, and other edible animal products, in comparison plants, appear to maintain a much greater constancy of composit as respects calcium, magnesium, and phosphorus. Hart, Steenbock a Kline ('30) were unable to note any effect on the per cent of c cium and phosphorus in milk of cows fed irradiated yeast. H and associates have also shown that cod liver oil is similarly witho effect. Even cows suffering from osteomalacia and showing a negat calcium balance, produced milk containing the same concentration calcium and phosphorus as normal cows (Becker, Eckles and Palm '27). This remarkable capacity to maintain constancy of composit in spite of adverse dietary circumstances, is well illustrated in the f that the calcium, magnesium, and phosphorus of eggs are scarc affected as long as the hen is able to make hard egg shells. Howev the production level of milk and eggs is greatly affected by dieta inadequacies.

REFERENCES

BESSEY, KING, QUINN and SHERMAN (1935). J. Biol. Chem., *111*, 115.
COX and IMBODEN (1936a). J. Nutr., *11*, 177.
GREENBERG and TUFTS (1936). J. Biol. Chem., *114*, 135.
SHERMAN and QUINN (1926). J. Biol. Chem., *67*, 667.
COLE and KOCH (1931). J. Biol. Chem., *94*, 263.
ARON and SEBAUER (1908). Biochem. Ztschr., *8*, 1.

RTIN (1937). Growth, *1*, 175.
LLING and ASHER (1932). Bull. Johns Hopkins Hosp., *50*, 318.
IPLIN and STEENBOCK (1933). J. Biol. Chem., *100*, 209.
IG (1931). Veterinary Med., *26*, 58.
BES (1937). J. Nutr., *14*, 419.
and MCCOLLUM (1938). Unpublished data.
LIS, DAY and MCCOLLUM (1938). Unpublished data.
ILER and GREEN (1932). Nutr. Abstr. & Rev., *1*, 359.
LES, GULLICKSON and PALMER (1932). Tech. Bull. No. 91. University Minnesota Agric. Exper. Sta.
LES, PALMER, GULLICKSON, FITCH, BOYD, BISHOP and NELSON (1935). ornell Veterinarian, *25*, 22.
EL, HUGHES and LIENHARDT (1936). J. Agric. Research, *52*, 149.
DY (1926). Compt. rend. Soc. de biol., *94*, 431.
COLLUM and ORENT (1931). J. Biol. Chem., *92*, xxx.
SE, ORENT and MCCOLLUM (1932). J. Biol. Chem., *96*, 519.
NT, KRUSE and MCCOLLUM (1932). Am. J. Physiol., *101*, 454.
NT, KRUSE and MCCOLLUM (1934). J. Biol. Chem., *106*, 573.
IN, ORENT and MCCOLLUM (1935). Am. J. Physiol., *112*, 256.
MER (1932). Lancet, *ii*, 174.
OKFIELD (1934). Brit. Med. J., *i*, 848.
TCHORN and MCCANCE (1937). Biochem. J., *31*, 1379.
ENBERG, LUCIA and TUFTS (1938). Am. J. Physiol., *121*, 424.
TS and GREENBERG (1938). J. Biol. Chem., *122*, 693.
MIDT and GREENBERG (1935). Physiol. Rev., *15*, 297.
ERSON (1920). Tech. Bull. No. 79, New York Agric. Exper. Sta.; J. ol. Chem., *44*, 429.
ABARGER, FREEMAN and IVY (1938). Demonstration, Federation of merican Societies for Experimental Biology, Annual Meeting, Baltimore.
RTNEY, TISDALL and BROWN (1928). Canad. Med. Assoc. J., *19*, 559.
KER and MATZNER (1923-24). Proc. Soc. Exper. Biol. & Med., *21*, 186.
, CRUM and LYMAN (1932). J. Biol. Chem., *95*, 29.
KE and SHERMAN (1935). J. Biol. Chem., *110*, 421.
MAN (1934). J. Am. Dietet. Assoc., *10*, 100.
KENZIE and MCCOLLUM (1937). Am. J. Hyg., *25*, 1.
and IMBODEN (1936b). J. Nutr., *12*, 509.
ENSTEN and MAYNARD (1934). J. Nutr., *8*, 715.
INS and SHEARD (1933). Proc. Staff Meetings Mayo Clinic, *8*, 249.
ES and KEITH (1914). Tech. Bull. No. 5, Ohio Agric. Exper. Sta.
OLLUM, HALPIN and DRESCHER (1912). J. Biol. Chem., *13*, 219.
LING (1932). J. Biol. Chem., *96*, 195.
NGTON and KAY (1926). Biochem. J., *20*, 777.
NGTON (1927). Biochem. J., *21*, 1187.
ANN (1933). Biochem. Ztschr., *262*, 3.
NE and SCHORMÜLLER (1934). J. Biol. Chem., *106*, 595.
IS and BUNKER (1935). J. Nutr., *9*, 301.
ANCE and WIDDOWSON (1935). Biochem. J., *29*, 2694.
and CALLOW (1934). Biochem. J., *28*, 517.

Lowe and Steenbock (1936). Biochem. J., *30,* 1126.
American Medical Association, Council on Foods (1937). J. Am. M
Assoc., *109,* 30.
Waltner (1927). Biochem. Ztschr., *188,* 381.
Brock and Diamond (1934). J. Pediat., *4,* 442.
Deobald and Elvehjem (1935). Am. J. Physiol., *111,* 118.
Guyatt, Kay and Branion (1933). J. Nutr., *6,* 313.
Goss and Schmidt (1930). J. Biol. Chem., *86,* 417.
Bills (1935). Physiol. Rev., *15,* 1.
Venar and Todd (1936). J. Lab. & Clin. Med., *22,* 221.
Taylor, Weld and Sykes (1933). Brit. J. Exper. Path., *14,* 355.
Morgan and Garrison (1933). Am. J. Physiol., *105,* 596.
Nicolaysen (1937). Biochem. J., *31,* 101, 107, 122.
Thomson and Collip (1932). Physiol. Rev., *12,* 309.
Shelling (1935). "The Parathyroids in Health and Disease." C. V. Mo
Co., St. Louis.
Wilder and Howell (1936). J. Am. Med. Assoc., *106,* 427.
McLean, Barnes and Hastings (1935). Am. J. Physiol., *113,* 141.
Shelling, Asher and Jackson (1933). Bull. Johns Hopkins Hosp., *53,*
Shohl and Wolbach (1936). J. Nutr., *11,* 275.
Kletzien, Templin, Steenbock and Thomas (1932). J. Biol. Chem.,
265.
Sherman (1937). "Chemistry of Food and Nutrition." 5th ed., The M
millan Co., New York.
Stearns (1931). Am. J. Dis. Child., *42,* 749.
Cox and Imboden (1936c). J. Nutr., *11,* 147.
Palmer, Eckles and Schutte (1928). Proc. Soc. Exper. Biol. & Med.,
58.
Cunningham (1933). New Zealand J. Sci. & Tech., *15,* 191.
Buckner, Martin and Insko (1932). Poultry Sci., *11,* 58.
Elmslie and Steenbock (1929). J. Biol. Chem., *82,* 611.
Day, Kruse and McCollum (1935). J. Biol. Chem., *112,* 337.
Hess (1929). "Rickets Including Osteomalacia and Tetany." Lea a
Febiger, Philadelphia.
McLean and Hastings (1935a). Am. J. Med. Sci., *189,* 601.
McLean and Hastings (1935b). J. Biol. Chem., *108,* 285.
Hoobler, Kruse and McCollum (1937). Am. J. Hyg., *25,* 86.
Hirschfelder (1934). J. Am. Med. Assoc., *102,* 1138.
Sjollema (1932). Nutr. Abst. & Rev., *1,* 621.
Duncan, Huffman and Robinson (1935). J. Biol. Chem., *108,* 35.
Day and Orent-Keiles (1938). Unpublished data.
Holt, LaMer and Chown (1925). J. Biol. Chem., *64,* 509, 567, 579.
Stewart and Percival (1928). Physiol. Rev., *8,* 283.
Robison (1932). "The Significance of Phosphoric Esters in Metabolism
New York U. Press, New York.
Shipley (1924). Bull. Johns Hopkins Hosp., *35,* 304.
Shipley, Kramer and Howland (1926). Biochem. J., *20,* 379.
Kay (1932). Physiol. Rev., *12,* 384.

ISON (1936). Ann. Rev. Biochem., *5*, 181.
DERSTRØM-LANG (1937). Ann. Rev. Biochem., *6*, 43.
ER, AUB and ALBRIGHT (1929). J. Exper. Med., *49*, 145.
EWITZ and HEVESY (1935). Nature, *136*, 754.
R (1932). Physiol. Rev., *12*, 593.
GINS (1935). J. Am. Med. Assoc., *104*, 1296.
TCH (1937). Nutr. Abstr. & Rev., *6*, 553.
CY, HUNSCHER, HUMMEL, BATES and POOLE (1936). J. Nutr., *11*, Suppl.,
NIELS, HUTTON, KNOTT, WRIGHT and FORMAN (1935). J. Nutr., *10*, 373.
BBELL and KOEHNE (1934). Am. J. Dis. Child., *47*, 988.
RMAN and HAWLEY (1922). J. Biol. Chem., *53*, 375.
D (1934). J. Home Economics, *26*, 605.
NS, SCHIEFELBUSCH, MARSHALL and COONS (1935). Tech. Bull. No. 223, Oklahoma Agric. Exper. Sta.
CY, HUNSCHER, MCCOSH and NIMS (1930). J. Biol. Chem., *86*, 59.
NELSON, NIMS, HUNSCHER and MACY (1931). J. Biol. Chem., *91*, 675.
NIS and TALBOT (1921). Am. J. Dis. Child., *21*, 29.
NIELS and EVERSON (1936). J. Nutr., *11*, 327.
RT, GUILBERT and GOSS (1932). Tech. Bull. No. 543, University of California, College of Agric.
HOP (1934). J. Nutr., *8*, 239.
EAVES and GREAVES (1933). J. Nutr., *6*, 113.
RT, STEENBOCK and KLINE (1930). J. Biol. Chem., *86*, 145.
KER, ECKLES and PALMER (1927). J. Dairy Sci., *10*, 169.

CHAPTER

VIII

Sodium, Potassium, and Chlorine

LOEB'S THEORY of physiologically balanced salt solutions has p
foundly influenced scientific thought in the field of nutrition. Fr
the fact that animal tissues respond so markedly to even slight chan
in ion concentration in the fluids bathing them, many investigat
have presumed that the proportions existing among the mineral e
ments in an animal's food are equally important to the maintenan
of health and physiological efficiency.

Since nearly all natural foods contain considerable amounts
sodium, potassium, and chlorine, it has been possible for man a
animals to subsist in many parts of the world, and in at least fai
good health, on the amounts of these elements supplied in their foo
In the Odyssey reference is made to inlanders who use no salt
their food. Benjamin Rush (cited by Kellogg, '23) stated that "althou
the interior parts of our continent abound with salt springs, yet
cannot find that the Indians used salt in their diet till they we
instructed to do so by the Europeans." The well-known fact that co
merce in salt was one of the major enterprises in ancient time attes
however, that mankind generally has always desired salt, and whe
it was obtained with difficulty it has commanded high prices.

Carnivorous animals do not develop a craving for salt (NaCl)
do Herbivora, a fact which may reflect the dietary sufficiency of th
element in the animals they eat. It may well be true that the likin
of Carnivora for blood is due in part at least to its salty taste. Grazin
animals crave salt, except in such areas as provide more than usu
amounts of sodium chloride in the water, and such places are not ra

Sodium, potassium, and chloride have been the subject of ma
investigations, particularly as relates to their ionic content in norm
and pathological tissues to "total base" content of body fluids an

sues, acidosis, acid-base equilibria, effects of the "level of intake," uresis, edema, etc. These subjects are fully discussed in such works Peters and Van Slyke's *Quantitative Clinical Chemistry*, in physio- gical chemistry texts by Bodansky, Hawk and Bergheim, and others, d will not be considered here. For discussion of the "ionic effects" the effect of ions on irritability and contractility, reference may be ade to any work on physiology. The discussion in this chapter will limited to the recent experimental studies on the effects of specific rvation for each of these elements, and, to the role of sodium and assium in the function of the adrenal cortical hormone.

Investigations dealing with the physiological role of sodium have en largely studies of the importance of salt, i. e., sodium chloride, her than the element sodium. Few definite experiments dealing with function of sodium alone have been made.

In 1873 Forster fed dogs on a low-sodium diet, extracting the food h water until very low in salts. Salt-free meat, starch, and lard were d as food. These dogs died more quickly than those which re completely starved. He explained this occurrence by the fact t urea is a diuretic and causes excretion of a larger volume of ne and probably more sodium; and the urea coming from the -free protein caused elimination of some sodium. He concluded t a dog will die if it does not get sodium. From our knowledge of rition today we can readily see that Forster had a multiple iciency.

In 1874 Bunge repeated the same experiment on pigeons and ob- ned similar results. Forster considered it salt starvation, but Bunge ught it was due to a lack of alkali needed to neutralize phosphoric sulphuric acids arising from the metabolism of proteins. Lunin, 1881, found that mice died in 3 days if they were totally starved, on a salt-free diet they lived on the average 23 days. These ex- iments indicated that we probably need sodium in our diet.

Dairy cows that do not have access to salt (NaCl) exhibit an ab- mal appetite for it in 2 or 3 weeks' time, but the health of the mals is not affected generally until a much longer time has elapsed bcock, '05). The breakdown is most likely to occur at calving or mediately after, when the system is weakened and the flow of k is large. In general, the cows giving the largest amount of milk the first to show signs of distress. The breakdown in health due lack of salt is marked by a loss of appetite, a generally haggard earance, lusterless eyes, a rough coat, and a very rapid decline in h weight and yield of milk. When these events follow calving in igh-producing cow, the collapse may be sudden and death may idly ensue. If given before the animal is in a desperate condition, ium chloride will effect a dramatically rapid recovery.

Cattle and sheep that have been deprived of salt (NaCl), gaining access to a salt lick, may consume sufficient salt to ca toxic effects, i. e., 4 to 8 oz. in the case of sheep and 1.5 to 5 for cattle. In acute cases the animals show extreme thirst, dep sion excitement, abdominal pain, and death in collapse. In less ac cases, profuse watery diarrhea which may become hemorrhagic in. Pregnant animals may abort. On post-mortem examinations, trointestinal inflammation and injection of the cerebral membranes generally found.

An excess of salt (NaCl) in the ration of chickens up to 8 cent of the ration excites no apparent detrimental effect on th condition, nor after they become accustomed to such salty ratio on their rate of growth (Mitchell, et al., '26). Baby chicks also exhi a high tolerance for salt (Quigley and Waite, '32).

Distribution.—Sodium, potassium, and chlorine are widely d tributed in nature. Potassium is especially abundant in both plant a animal tissues. It is the chief mineral constituent of muscle and most other tissues, very little being present in the fluid portions the organism. This element is especially utilized by newly formed a growing tissues. Practically all foods of vegetable or animal orig contain several times as much potassium as sodium. The greater pa of the sodium in the ordinary daily diet is added as seasoning the form of sodium chloride, while the potassium is furnished the various organic and inorganic potassium salts of the animal an vegetable tissues employed in the diet.

In the blood of man, sodium predominates in the plasma an potassium in the red corpuscles. (Na-43 mg./100 cc. corpuscle 335 mg./100 cc. serum. K about 425 mg./100 cc. corpuscles; 18- mg./100 cc. serum.) This is also true for the rat, rabbit, guinea pi pig, and monkey. In the cat, dog, sheep, and cow, sodium and pota sium concentrations in the red cells tend to approximate the conce trations of these ions in the serum. Sodium is the predominant ba in lymph, edema fluid, cerebrospinal fluid, and other extracellul fluids. Cartilage is especially rich in this element. The whole huma body has been estimated to contain 0.1 per cent sodium and 0.11 p cent potassium.

Vegetable foods are poor in chlorine but this element is foun in all the tissues of the animal organism. The concentration of chlori in normal serum varies from about 362-376 mg. per 100 cc. It h generally been assumed that it all exists in the body in inorgan form. However, Hanke and Donovan ('27) stated that 15-20 per ce of the total chlorine of the body is in combination with lipoid su stances. Cameron and Walton ('28) could not confirm these resul

Absorption and Excretion.—Sodium, potassium, and chlorine, un- normal conditions, are easily absorbed from the intestinal tract l are readily excreted by the kidneys. Although little of an exact ure is known concerning the mechanism by which the excretory ans function in selecting the ratios of these elements for excretion, s clear that they do so. Accordingly, there is maintained in the ues and body fluids a fairly constant concentration.

Excessive ingestion of any salts leads to increased thirst and in- ased output of urine or sweat or both, which carry away the sur- s. Ordinarily sweat contains 0.7 per cent sodium chloride. On an rage man excretes 10 gm. sodium chloride in the urine and sweat ly which indicates that 10 gm. of the salt are consumed in the d, since normally the intake is just balanced by the output. Dur- starvation the body retains the sodium chloride very tenaciously. a metabolism study conducted at the Carnegie Nutrition Labora- on a man who fasted for a month, it was found that the ount of sodium excreted progressively decreased from 2.5 gm. to gm. on the tenth day. On administration of 10-15 gm. sodium ride per day, it took 4 days for the excretion to rise above 0.1 gm. day. The body evidently was retaining practically all of the salt eplenish the deficiency. Dill and others ('33) state that not more n 15 gm. of sodium chloride can be excreted daily in sweat. Heller) found that with salt solutions for drinking water the upper rance limit was 1.5 to 1.7 per cent (NaCl) and animals died of st rather than drink such solutions. In many dry regions the er holes contain excessive amounts of sodium chloride and car- ate, and the forced drinking of such waters may adversely affect health of animals. Mistaken views have arisen in this connection, s illustrated by the belief of farmers in the Middle West that the alled "alkali disease" was due to too much salts in the water. Later overies showed that the condition was selenium poisoning.

Gompel et al. ('36) found that rabbits consumed with reluctance l containing 1 per cent sodium chloride, and rejected it when the ent was raised to 2.5 per cent. By gradually increasing the con- of sodium chloride, rabbits could acquire tolerance and readily food containing 3.5 per cent (NaCl). When the concentration eded 0.4 per cent the water consumption increased in proportion he salt concentration to such an extent that the final concentra- of salt in the food was less than 1.3 per cent. There were some vidual variations. Human subjects differ in their taste for salt their food.

General Functions.—Although we know that sodium, potassium, chlorine are indispensable in the diet, our knowledge of their

function and importance in metabolism is fragmentary. The func of the minerals are so manifold and interrelated with so many processes that the mechanism of their actions is still obscure. Min are part of every living cell. The chemical processes that charact living matter take place in salt solutions. Osmotic pressure mus maintained within precise limits for the proper behavior of the in the mammalian organism. The maintenance of this osmotic sure, as well as the maintenance of physiological neutrality, dep primarily on the total base content in both intra- and inter-cell fluids.

The principal basic elements, calcium, magnesium, sodium, potassium, influence many properties and functions of living cells cluding water content, osmotic pressure, permeability, irritability, tractility, secretion, excretion, and general metabolism. The irritab of the nervous system is dependent in a large measure upon the ance between the amount of these ions present in the tissues and b fluids. The maintenance of certain relationships among the individ bases appears to be necessary for proper muscle irritability.

Chlorine assists in the maintenance of physiological neutrality the body fluids and plays a part in the distribution of water in blood, the intercellular tissue spaces and serous cavities, and the b cells. Presumably the tissue cells in general swell and shrink as the blood cells with changes in the salt content of the fluid bath them and suffer if such changes are too great.

The Effects of Sodium Deficiency.—On the basis of their stud with diets containing very low concentrations of each of several the essential inorganic elements, Osborne and Mendel ('18) conclud that "the law of the minimum" holds for all essential salts in the d and that failure of growth in the body as a whole results when limiting factors are deficiencies of sodium, chlorine, potassium, m nesium, calcium, and phosphorus. They found that young rats gr normally on a ration containing but 0.035 per cent of potassium sodium.

Miller ('26) secured satisfactory growth on a ration containi 0.07 per cent of sodium, and observed no deleterious effect of a ra of K: Na 14: 1. He did not get normal growth when the sodi in the diet was reduced to 0.03 per cent, but growth was satisfact when a sodium supplement was provided. Richards and associa ('24; '27) found that a diet composed of corn, oats, barley, and blo meal was improved as respects retention of nitrogen, calcium, a phosphorus, by supplementing it with sodium citrate or chloride. Si ilarly, Mitchell and Carman ('26) employed a ration for rats a chicks consisting largely of corn, and found it was below the op mum in both sodium and chlorine, and that its deficiency of sodi

s probably greater than of chlorine. Their diet contained 0.047 per
t of sodium and 0.041 per cent of chlorine. They found deficiency
these elements to limit the utilization of protein and energy for
wth. The digestibility of protein on the unsupplemented ration was
impaired by deficiency of hydrochloric acid in the gastric juice,
l the effects of the deficiency of chlorine seemed to be related
re to the requirements of growth than to those of gastric digestion.
In 1928 St. John, using a somewhat better diet (containing 0.3%
ium) than the other investigators, demonstrated retarded growth
l abnormal reproduction, and also noted that the eyes of his ani-
ls were affected, blindness resulting in many cases.
In 1934 Schoorl studied the influence of sodium depletion on growth
l other physiological constants in rats and pigs. He also noted a
reased rate of growth. He states that fertility is not affected but
roduction is impossible on his diet because owing to the disturbed
tein metabolism, milk secretion is impaired.
Sjollema ('35) observed that the growth of chicks was considerably
rded by a ration poor in sodium.
In paired feeding experiments, Kahlenberg, Black and Forbes ('37)
ployed a corn-supplemented ration and observed that sodium de-
ency unfavorably affected appetite, growth, storage of energy, and
thesis of fat and protein. The proportion of energy stored as fat
larger with the sodium-supplemented than with the sodium-defi-
t diet. There was no impairment of digestion, and the water content
he bodies of their animals was normal. Heat loss was significantly
her in the sodium-deficient rats than in the supplemented ones.
diet used in this investigation, although containing but 0.03 per
sodium, appear to be inadequate in some of the necessary dietary
stituents, especially the vitamin B complex.
While the above-mentioned experiments have elucidated to some
nt the importance of sodium, the diets used contained considerable
unts of this nutrient and almost without exception they were obvi-
y deficient in some other dietary essentials. Nevertheless, these
ies are important since they show, among other things, that a
al diet is likely to provide too little sodium for maximum rate
levelopment and utilization of food. They justify the addition of
mon salt to diets largely derived from cereals.
Orent-Keiles, Robinson and McCollum ('37) restricted young rats
a diet composed of acid-washed and alcohol-extracted casein, a
hyl alcohol-gaseous HCl extract of yeast, sucrose, sweet butter fat,
mixture, and viosterol. This diet contained 0.002 per cent of
um, and was otherwise complete as was shown by its satisfactory
racter when supplemented with sodium salts. Ninety experimental
50 control rats weighing 35 to 40 gm. were employed in the

investigation. In other experiments with the same diet crystallin min, purified riboflavin, and B_6 in the form of Peter's eluate used as the vitamin B-complex instead of the yeast extract. T sults in this set of experiments were similar to the ones whe yeast extract was used. The animals on the sodium-low diet gr more than half the normal rate for a few weeks, after which began to lose weight. No striking symptoms were noted unt 6th to the 8th week. At this time the sodium-low animals beg show eye changes. Some exhibited bluish-gray corneae; in other corneae were still normal at this time; some had perforated u and all showed a thin sanguinolent excretion covering both eyes.

During the next 2 weeks practically all the rats developed th lowing eye changes: thickening and bluish-gray corneae, sanguin secretion, corneal ulceration, perforation of the ulcers, hypop marked bulbar and ciliary injection, edema of the lids, loss of of lids, loss of lashes, and abscess of the anterior segment of the

At the end of the 8th week of the experiment, 19 animals selected for histological studies of the eyes. They were divided groups A and B. Those in group A were killed; their eyes were moved and prepared for histological examination. Those in grou were continued on the sodium-low diet. These rats died in the week of the experiment, all showing the symptoms described ab The eyes of these animals were also studied histologically.

Microscopic examination of the group A animals showed cor lesions with extensive anterior synechiae, also posterior synechiae beginning capsular cataract. Wandering cells were present in the terior chamber. In others, the corneal epithelium was thickened places and showed areas of ulceration. The entire thickness of cornea was infiltrated with wandering cells of all types and th was apparently perforation, for the iris was adherent to the cente the cornea in this region. There were some round cells on the face of the iris in the posterior chamber. The lacrymal glands appea to be normal.

The histologic examination of the rats in group B, after 20 we on the sodium-low diet showed the following changes: in a num there was intense infiltration throughout the cornea which had dently been associated with perforation of ulcers inasmuch as the was adherent to the back of the cornea over its whole extent. corneal epithelium was composed of thin basal cells which were tened. The superficial two-fifths were keratinized; in certain anin there was intense purulent keratitis with perforated ulcer and hem rhage into the anterior-chamber. In places the corneal epithelium greatly thickened. Basal cells were irregular in pattern and the su ficial layer was keratinized. There was a thick plaque of hyaline m

over the ulcer which contained colonies of bacteria. The sections the control rats' eyes were all normal.

These eyes differed from those of vitamin A deficient rats in that re was no diminution in secretions of tears. In A deficient rats the s are dry owing to keratinization of epithelium in the lacrymal nds. In the sodium-low animals there was at least normal tear duction and in some instances it appeared to be excessive.

In the earlier literature on xerophthalmia in vitamin A deficient , several observers called attention to ulceration of the cornea and opyon in animals in this deficient state. Wolbach and Howe ('25), o made the most thorough study of the pathology of this state, ert that they "have not a single example of ulceration of the cornea of hypopyon" in their series of animals. It is apparent, therefore, t the eye disturbances of the rat induced by deprivation of sodium er in almost all details from those caused by vitamin A deficiency. When killed, the sodium-deficient rats appeared almost free from deposits, the muscles were atrophied, the liver a darker reddish wn than normal, and with some mottling. The spleen was very k and greatly reduced in size. The kidneys were abnormally dark showed occasional mottling. The adrenal glands were orange rather n pink in color. The testes appeared atrophic. The bladder was ally filled with urine and the stomach and intestines were dis- led with gas. All animals showed lung infections.

The bones of the sodium-deficient rats appeared retarded with re- t to growth and they were fragile. There was a deficiency of ilage and osteoid tissue.

A study of the estrual behavior showed that sodium-deficient rats ured about the 66th day of life as contrasted with the 55th day in controls. Daily vaginal smears demonstrated that the experimental nals exhibited an essentially normal ovulatory performance during verage period of 14 days. Thereafter a characteristic disturbance observed in the depleted animals which resembled that of vitamin eficiency. There was prolongation of the estrus desquamation stage he vaginal epithelium; the smears showed almost exclusively large, , cornified cells which normally characterize the actual period of s and ovulation. In these animals this occurred throughout the e depletion period. In addition to cornified cells there were pres- small amounts of mucus; in some cases excessive numbers of cytes; and in others, a few cornified, nucleated epithelial cells. itions of vitamin A in the forms of butter fat and carotene respec- were tested as supplements to that already present in the diet. epair of the vaginal epithelium did not occur following the ad- stration of large doses of this vitamin.

Twelve sodium-depleted female rats, separately caged, were placed

during each working day with a stock male, during a ten-week per
Postmortem examinations showed no signs of pregnancy in the
of 8 animals; 1 contained a single fetus, the other contained 5 fet
all in one uterine horn. One fetus weighed 4.5 gm., another 2.2
and the remaining 3 had a collective weight of 1.7 gm. The si
fetus in the other rat weighed 3.6 gm.

Sperm motility tests by means of testicular smears, gross exam
tion of testes at autopsy, and mating tests, were made on a se
of 12 males. Motility of sperm remained normal until about the
day when in 5 animals there was a decrease in motility. The
didymides appeared to be somewhat atrophied, and there seemed
be a decrease in their fluid content. Although the testes were sma
than those of the controls they were normal in proportion to b
weight, and their appearance was essentially normal.

Six other males were used for mating tests using vigorous st
females on which vaginal smears were made regularly to determ
their regularity of ovulation. It was shown that these males remai
fertile for at least 75 to 80 days on the sodium-low diet.

In a study of the metabolism of sodium-deficient animals Scho
('34, l.c.) carried on protein balance experiments and reported t
the decreased growth of rats and pigs fed a sodium-low diet is
result of an increased nitrogen metabolism. He found that the grea
part of nitrogen was excreted in the form of urea. His sodium-defici
animals did not exhibit any signs of uraemia in spite of the increas
production of urea. He concludes from his findings that sodium w
needed in the diet to enable the organism to utilize the resorbed p
tein for building up body protein.

Sjollema ('35, l.c.) showed that the excreta of sodium-defici
chicks were rich in nitrogen and contained but traces of sodiu
However, the birds were in positive sodium balance throughout t
duration of the experiment.

Metabolism balance studies on the sodium-low diet devised
this laboratory are in progress now, using the paired feeding techn
Analyses of the urine and feces of the sodium-deprived rats duri
the first 6 weeks of the experimental period (18-21 weeks) show p
gressive changes in the phosphorus and nitrogen retention. T
sodium-deficient rats begin to show negative balances early in t
experiment and by the 6th week on the diet all of the experimen
animals are in negative nitrogen balance. Thus the increased p
tein metabolism explains the decreased rate of growth of the r
deprived of sodium. The sodium excretion of the animals on t
sodium-deficient diet is at a very low level from the first week
the experiment, being 2 to 7 mg. in contrast to 86-124 mg. of t
controls. Hence, the sodium-deficient animals are consistently in ne

sodium balance. In case of phosphorus, during the first week a itive balance is exhibited. At the end of the second week it is positive but to a lesser degree. From the third week on negative nces begin to appear and by the end of the 5th week all the ium-deprived rats are in negative phosphorus balance. The cal-m, magnesium, potassium, and chlorine metabolism is at this time not disturbed. Similar analyses of the excreta of control animals, those receiving the sodium-low diet supplemented with sodium, w no abnormal changes in the metabolism of these constituents.

Effects of Potassium Deficiency.—In their physico-chemical be-ior sodium and potassium are practically alike, so far as the tions in which they are known to take part are concerned. Why play such different roles in the organism we have not the ntest idea. Thus, although it has long been recognized that potas-n is essential for animal life, the effect of a deficiency of this nent on animals is at present unknown. Very little work has been e on the study of the symptomatology of potassium deficiency, oubtedly due partially to the difficulties in preparing a diet free n or low enough in potassium to furnish this element in smaller unts than the minimum requirements.

Osborne and Mendel ('18, l.c.) restricted rats to a diet containing 3 per cent of potassium and otherwise adequate. The growth was rded somewhat more than when sodium was reduced to 0.035 cent but the animals continued to gain slowly for over 300 days. sodium, potassium is held tenaciously when the supply is very ted.

Miller ('23) reported a very slow growth and an abnormal alert-as the two principal symptoms of rats fed a purified diet low otassium. Leulier and Vanhems ('34) found that young rats to grow, and die in 12 to 24 days when fed a diet deficient in ssium.

The effects of deficiency of potassium in rats have been described Schrader, Prickett and Salmon ('37). Their potassium-deficient consisted of an extracted grain mixture supplemented with puri-casein, cod liver oil, and a salt mixture free from potassium. grain mixture was composed of yellow cornmeal and wheat mid-gs and was extracted with 0.2 per cent acetic acid for 6 days with distilled water for 1 day. In certain experiments the diet supplemented with 10 mg. of a fuller's earth adsorbate, found igeon assay to contain 3 I. U. of thiamin. Their records indicate this addition was without effect on weight changes. On this they observed that young rats made no growth and the aver-loss of weight during the average duration of life, which was ays, was 10 per cent. They describe the symptoms as early leth-

argy, with progressive increase of abdominal distention; pale with cyanosis; and pendulous sacs in the region of the thyroid g and sternum. At necropsy severe ascites was usually present, an a few cases there was hydrothorax and hydropericardium. Atony transluscence characterized the intestine, and in the ileum there thickened annular areas about Peyer's patches, showing conges Extensive pathological changes in other tissues were also descri

Unfortunately the diets of these different workers are obvio not adequate in the other dietary essentials aside from potass The diet of the last mentioned group was particularly unsatisfact therefore it cannot be accepted that the observations recorded by t investigators are actually due to potassium depletion of the an organism.

Orent-Keiles and McCollum ('38) have observed the effects deprivation of potassium in rats. Their results are so completely variance with those of Schrader and associates (l.c.) that it is cluded that the latter did not produce in their animals a state potassium starvation alone but a syndrome caused by deficiency some dietary factors other than this element. Orent-Keiles emplo a diet consisting of purified lactalbumin, wheat gluten, gelatin, mixture, sweet butter fat, dextrose, the vitamin B complex, viost and vitamin E concentrate.

With the exception of potassium, this diet was complete in other respects as shown by the fact that in rats on the experimen diet plus potassium essentially normal growth occurred, ranging fr initial weights of 35-40 gm. to 240-288 gm. at 8½ months, after wh increment of growth became slower. These control rats have rep duced and successfully weaned several litters of young. Such effe as were observed in the experimental group are, therefore, to be tributed solely to potassium deprivation.

Young rats on the potassium-low diet grew very slowly but ste ily and at 8½ months were still increasing 1 to 4 gm. per we This result is comparable to but more pronounced than that Osborne and Mendel ('18, l.c.) whose diet contained 0.033 per c of potassium, or about 2.5 times the content of this element p vided by the Orent-Keiles diet. In the potassium-low animals the ti of sexual maturity and ovulation was retarded. The vaginal orif was dry and the insertion of a thin glass rod was difficult even af the vagina had been open for some time. There was disturbance the estrual rhythm. When mated with stock males no impregnatio occurred. Control females produced and raised several litters of you In the males, testicular smears showed that the reproductive syst was affected. The motility of the sperm cells was reduced marke and many spermatids were present. Later the smears showed de

n and spermatids. Males, when mated with normal females, were d incapable of fertilizing them. In both sexes the fur was thin somewhat rough. The animals were extremely alert and active, biting an unrest which was strikingly apparent. They also manid a form of pica which caused them to almost constantly search t the cage and lick various parts of the cage and its equipment; ck each other, particularly their genital orifices, especially after ation.

he rate and rhythm of the heart beat in potassium-low rats was tigated by means of the electrocardiograph. The potassium-defi- rats show a slowing in the heart rate and irregularity in the am.

reliminary histological studies of rats deprived of potassium show there is a characteristic scarring of the heart muscle, the kid- increase in weight, and the bones are fragile and retarded in th.

he probability of a deficiency of potassium in the human or ani- diet under normal conditions is extremely remote, for the ubiqs occurrence of this element in plant and animal tissues used odstuffs and the small amount of the mineral required, guar- against a deficit of this base.

owever, in the light of the observations recorded, the treatment tients with Addison's disease by the present practice of restrictnem to a diet low in potassium, especially over a prolonged period ne, may be fraught with some hazard. As yet no data are availon which to base judgment as to the minimum intake of this nt by man which is consistent with safety, but caution may be able in planning low potassium diets to be used over long ds of time.

ffects of Chlorine and Sodium Chloride Deficiency.—Experis dealing with the depletion of the organism of the chlorine ion are but few. Rosemann ('11) observed that when the diet is ent in chlorides there is practically no depletion of the body nt of chlorine (chlorine ions) since excretion decreases at once practically ceases. Osborne and Mendel ('18, l.c.) fed to rats a containing but 0.035 per cent of chlorine. Starting at a body t of 90 gm., one of the rats was but little retarded in growth it reached a weight of 220 gm. Thereafter it continued to grow slowly to a weight of nearly 300 gm. From then on it grew more slowly. However, it was still able to respond with rapid h when placed on a normal diet. They noted that it could ave been expected that on a diet so low in chlorine the rats l be able to continue to thrive so long or to attain so many their original weight. This study showed that animals receiv-

ing but extremely small amounts of chlorine husband practica of the quantity ingested.

Orent-Keiles, Robinson and McCollum ('37, l.c.) restricte to a diet practically free from chlorine and found that they somewhat retarded in growth, but showed no other symptom ing a 90-day period. Animals on a diet deficient in both sodiu chlorine grew more slowly than did those deficient in chlorine After the third week rats deprived of sodium chloride becam nuded of hair about the back and shoulders. They appeared apprehensive. At the end of the first month the mouth, ears, and forepaws were bloody and the urine was dark brown in Denudation increased as the experiment proceeded but was rest to the anterio-dorsal parts of the body. The sodium chloride-de rats showed evidence of internal hemorrhages in the thymus liver. The testes were small in proportion to the size of the an but otherwise appeared normal. The adrenals were smaller than of the controls. The bones were not fragile as were those of soc depleted rats.

The only circumstances under which normal human subject observed to suffer a deficiency of sodium chloride are where are exposed to the excessively high temperatures and low humidi in furnace rooms and mines. Men working under these condi lose salt to an extent which causes "heat cramps" or "bends." condition is prevented by drinking water containing sodium chlo Excessive amounts of sodium chloride and sodium bicarbonate also be lost through diarrhea.

The Role of Sodium and Potassium in Carbohydrate Met lism.—Chaikelis ('34) showed experimentally that there is an in ratio between blood chlorides and blood sugar, and that this is a compensatory mechanism concerned with the maintenance of mal osmotic relationship. He expressed the view that this chang blood chloride is associated with some phase of carbohydrate me lism. Crabtree and Longwell ('36) have studied this problem fur and found that when young rats were fed the Osborne and Me ('18, l.c.), "chloride-free" diet, there was no effect on the liver glyc stores. With the same diet fortified with 6.25 per cent of soc chloride, and distilled water *ad libitum,* the glycogen content about one-third higher than in the "chloride-free" livers. There no significant difference in the muscle glycogen in the two gr

These observations are of special interest in connection with studies of McQuarrie and associates ('36) on the craving of yo diabetic subjects for salt (NaCl) and the influence of excessive ingestion on carbohydrate metabolism. The daily ingestion of 2 gm. of sodium chloride per kilogram of body weight resu

in 2 to 4 days in a gain of 4 to 5 per cent in weight and in ease in both systolic and diastolic blood pressure to new plateaus reen 30 and 40 per cent above the control levels. These higher sures were maintained so long as the salt was taken. In addi- to these there was marked reduction in the degree of glycosuria. effects were qualitatively the same in one mildly diabetic pa- not receiving insulin, as in diabetic patients who received insulin hour intervals. Other conditions being unchanged, the fasting d sugar remained at lower levels after a few days of a high um chloride intake. In severe diabetes, ketosis appeared to de- o earlier after the withdrawal of insulin when the sodium chlo- intake was low than during the periods of high intake.

atients maintained on a constant protein intake estimated to ntain nitrogen balance were found to be in negative nitrogen nce during periods of low salt feeding, but retained nitrogen when sodium chloride intake was high. Typical insulin reactions were mes observed during periods of high sodium chloride intake atients receiving insulin in doses sufficient to prevent glycosuria ng low-salt periods.

odium bicarbonate and sodium citrate, when given in amounts valent to the sodium values, in cases of sodium chloride admin- ion, had similar though less marked effects than did sodium ide. The maximum effects of sodium chloride on both blood ure and carbohydrate metabolism were obtained only when patients were given simplified diets low in potassium. Ordinary high in potassium either prevented or greatly lessened the ef- of the sodium chloride. When potassium chloride was given in of 10 to 20 gm. daily along with the therapeutic diet low in m chloride, the blood pressure was lowered, and at times a ficant increase in the glycosuria recurred. In these respects, there- there is shown a physiological antagonism between sodium and sium, comparable with that which has long been known in rela- o muscle irritability and contractility. In the studies of McQuarrie pears that one part of potassium counteracts the effect of at three equivalent parts of sodium. High sodium chloride intake, ound, depresses significantly the potassium content of blood n. In view of these observations, it is thought that a low-sodium, potassium regime might prove to be beneficial in cases of spon- us hypoglycemia.

elation of Sodium and Potassium to Adrenal Function.—Of ular interest are the recent observations on the relation of the al hormone of the adrenals to sodium and potassium metabolism. most striking feature of experimental and clinical adrenal in- ency and one for which an explanation at this time is unavail-

able, is the metabolism of sodium and potassium. During the
of the syndrome the sodium of the blood is lowered and the
sium is increased. The condition may be corrected by the ad
tration of sodium salts and cortin singly or in combination. Ho
it has been found difficult to maintain adrenalectomized anima
patients who have Addison's disease, in an entirely normal co
and with a normal blood electrolyte pattern by supplying s
salts alone. The increases in the serum potassium in these cas
not necessarily parallel to the degree of depletion of sodiu
may at times be present even when other electrolytes of the
have been returned to normal by the administration of sodium
Allers ('35) and Kendall ('35) were the first to show that adre
tomized animals could be thrown into crises of adrenal insuffi
by the addition of potassium to their diet, even while adequate
plies of sodium salts were provided, and conversely, that a low
of potassium in the diet was of great importance in maintaining
animals in optimal condition with normal blood electrolytes.
studies have been confirmed and extended by Zwemer and T
kowski ('36) who also have demonstrated the extreme sensitivi
adrenalectomized animals to potassium salts. These investigators
produced the symptoms of adrenal insufficiency in normal an
by raising the plasma potassium by means of repeated intraperit
injection of potassium chloride. Such studies seem to indicate
the role of cortical hormone depends essentially on its functio
regulating the metabolism of sodium and potassium.

Loss of sodium in the adrenalectomized animal or in case of A
son's disease is accounted for by Vérzar and Laszt ('36) on the
of inhibition of glucose absorption due to lack of cortical horm
The sugar remains in the intestine, causing a transfer of water
the tissues by osmosis, and of sodium by diffusion. When soc
salts are included in the diet this diffusion is prevented.

Observations on patients with Addison's disease demonstrated
a low intake of potassium will protect the patient to a consider
extent against withdrawal of sodium salts. A high intake of soc
alone does not suffice to prevent a crisis of adrenal insufficiency
the therapy of Addison's disease it is of greater importance to re
the potassium in the diet than to administer high doses of soc
chloride and the best results are obtained if both of these meas
are used. When such a regime is used, the patients require less co
and sodium salt and are less subject to relapse.

The preparation of a palatable potassium-low diet (Wilder, et
'36) requires the same careful planning as does that of a diet
a diabetic patient. The potassium values of foods, in percentag
the edible portion, that are available at present were compiled f

ous sources and for this reason are not the most satisfactory, for e is a considerable variation in the potassium content of foodstuffs ined from different regions due to the soil composition, fertilizer tment, etc.

Relation of Potassium to Familial Paralysis.—The effect of potas- n on certain types of paralysis is of interest. During the past year ral reports have been recorded on the control of familial periodic lysis with potassium salts. Familial periodic paralysis is a rare ase characterized by periodic abrupt attacks of flaccid paralysis, out sensory loss or psychic disturbances of any sort and from h recovery is complete. In about 80 per cent of the cases the se is hereditary. It is transmitted by both sexes, but males are ted twice as frequently as females. The etiology of the disease is nown. The attacks begin in most cases at puberty. The muscles ne limbs are chiefly affected, but in severe cases the abdominal respiratory muscles are also attacked. The paralysis lasts from w hours to a few days, or even longer. The attacks vary in fre- ncy, occurring every few days or at intervals of months or years. lyses of the blood during and between the paralytic attacks ed that the onset of paralysis was associated with a fall of n potassium from the normal value of about 19 mg. to below g. per 100 cc. serum. The paralysis can be relieved in 15 minutes he ingestion of potassium salts such as potassium chloride or ssium citrate (Mitchell and associates, '02; Aitkin, et al., '37; Her- on, '37; Gammon, '38).

ammon (l.c.) observed no excessive elimination of potassium by kidneys preceding the seizures. At the time of the attack the rption of potassium from the gut was quite low. Administration otassium salts prevented the development of seizures or prevented from occurring as frequently or in as severe a form. This in- gator concludes that such patients have an unusual need for sium salts to maintain contractility of certain of their muscles. ther bases involved in muscle metabolism such as sodium chlo- sodium bicarbonate, creatine hydrate, and prostigmin are ineffec-

hese observations, suggesting a relationship of disturbed metabo- to this disease, are of interest for they promise to illuminate this rto obscure malady.

elation of Potassium to Intestinal Stasis.—Robertson ('37) after ving that rats restricted to a diet low in mineral elements suf- intestinal stasis accompanied by dilatation and overloading of arge intestine with fecal matter, made a detailed investigation e effect of inorganic salts on intestinal elimination. She found stasis could be cured by supplying the missing elements. The

condition is relieved, or prevented, by supplying in the diet
priate amounts of calcium and potassium. The contents of the
intestine is definitely more acid in stasis due to deficiency of
elements than in the controls receiving calcium and potassiu
a series of 19 children who were fed diets low in these ele
14 showed constipation. Her studies suggest that the modern h
diet, consisting to a large extent of refined cereal products and
and providing far less total mineral elements than were con
in diets before the advent of modern methods of milling and
manufacture of sugar, may be in some measure responsible fo
prevalence of constipation.

Dietary Requirements of Sodium, Potassium, and Chlor

Very little information is available concerning the requiremen
man and animals for potassium, sodium, chlorine, or sodium chl

From the growth and general physiological behavior of
subsisting upon rations of varying mineral content, South Af
investigators (Theiler, Green and DuToit, '27) have concluded
the requirements of growing cattle for sodium and chlorine are
about 1.5 gm. daily of sodium and less than 5 gm. of chlorine.
ing lactation the sodium requirement of a cow (DuToit, et al., '3
11 gm. per day, and 15 gm. of chlorine daily suffices for the
animal at this time.

In the case of poultry 0.5 per cent sodium chloride in the
appears to meet the requirements of either the growing chick
the laying hen judged by growth, egg production, and mort
(Halpin, et al., '36).

It is of interest to note that the character and composition of
rations fed affect in large measure the sodium chloride consump
and requirements of fattening lambs (Evvard and associates,
Feeding beet molasses markedly decreased salt consumption, whe
alfalfa had the opposite effect. Also, the greater the proportio
roughage in the ration, the larger the sodium chloride consump

Man consumes 3-6 gm. of sodium in the ordinary daily diet,
greater part of which is supplied by the sodium chloride adde
seasoning.

With regard to the potassium requirement, 25 grams of potass
daily seems to be more than adequate for calves through gro
and gestation, while 32 gm. suffice during the period of lacta
(DuToit and associates, '34, l.c.).

The ordinary diet of man furnishes 2-4 gm. of potassium w
comes from the various animal and vegetable tissues used as f
It is thought that 0.06 gm. of potassium per kilo body weight su
for the normal adult, but that the nursing child requires 0.07
per kilo body weight for its growth. In early life the rapid gro

ne tissues requires potassium, and much of the potassium ingested
ne food is retained. After the age of 12 years the retention gradu-
grows less and only that amount which may be regarded as
e or less the normal requirement is held by the cells. However,
data on the minimum physiological intake of potassium, as far
an is concerned, are not very definite.

REFERENCES

I (1923). Cited by Kellogg, "The New Dietetics." p. 471, The Modern
edicine Publishing Co., Battle Creek, Mich.
TER (1873). Ztschr. f. Biol., *9*, 297.
GE (1874). Lehrbuch der physiol. Chem., *4*, Aufl., 97.
N (1881). Ztschr. f. physiol. Chem., *5*, 31.
OCK (1905). Wis. Agr. Exper. Sta. Ann. Reports, p. 129.
HELL, CARD and CARMAN (1926). Ill. Agr. Exper. Sta. Bull., *279*, 135.
LEY and WAITE (1932). Md. Agr. Exper. Sta. Bull., 340, 343.
KE and DONOVAN (1927). Proc. Soc. Exper. Biol. & Med., *24*, 580.
ERON and WALTON (1928). Trans. Roy. Soc. Canada, *22*, Sec. V, 1.
, JONES, EDWARDS and OBERG (1933). J. Biol. Chem., *100*, 755.
LER (1932). J. Nutr., *5*, 421.
PEL, HAMON and MAYER (1936). Ann. Physiol. et Physico.-Chim. Biol.,
504.
RNE and MENDEL (1918). J. Biol. Chem., *34*, 131.
ER (1926). J. Biol. Chem., *70*, 759.
ARDS, GODDEN and HUSBAND (1924). Biochem. J., *18*, 651.
ARDS, GODDEN and HUSBAND (1927). Biochem. J., *21*, 917.
HELL and CARMAN (1926). J. Biol. Chem., *68*, 165.
OHN (1928). J. Biol. Chem., *77*, 27.
ORL (1934). Proc. Roy. Acad. Sci. Amsterdam, *37*, 239.
LEMA (1935). Biedermann's Zentralbl., Abt. B. Thiernähr., *7*, 184.
LENBERG, BLACK and FORBES (1937). J. Nutr., *13*, 97.
T-KEILES, ROBINSON and MCCOLLUM (1937). Am. J. Physiol., *119*, 651.
BACH and HOWE (1925). J. Exper. Med., *42*, 753.
ER (1923). J. Biol. Chem., *55*, 61.
IER and VANHEMS (1934). Compt. rend. Soc. de Biol., *115*, 57.
ADER, PRICKETT and SALMON (1937). J. Nutr., *14*, 85.
T-KEILES and MCCOLLUM (1938). Unpublished data.
MANN (1911). Arch. f. d. ges. Physiol., *142*, 208.
KELIS (1934). J. Biol. Chem., *105*, 767.
TREE and LONGWELL (1936). Proc. Soc. Exper. Biol. & Med., *34*, 705.
CARRIE, THOMPSON and ANDERSON (1936). J. Nutr., *11*, 77.
KS (1935). Proc. Staff Meet. Mayo Clinic, *10*, 406.
ALL (1935). Proc. Staff Meet. Mayo Clinic, *10*, 408.
IER and TRUSZKOWSKI (1936). Science, *83*, 558.
AR and LASZT (1936). Nature, *138*, 844.
LR (1936). Proc. Staff Meet. of the Mayo Clinic, *11*, 281.

Wilder (1937). Archiv. Internal Med., *59*, 367.
Mitchell, Flexner and Edsall (1902). Brain, *25*, 109.
Aitkin, Castleden and Walker (1937). Clin. Sci., *3*, 47.
Herrington (1937). J. Am. Med. Assoc., *108*, 1339.
Gammon (1938). Proc. Soc. Exper. Biol. & Med., *38*, 922.
Robertson (1937). Am. J. Dis. Child., *53*, 500.
Theiler, Green and DuToit (1927). J. Agric. Sci., *17*, 291.
DuToit, Malan and Groenewald (1934). Onderstepoort J. Vet. Sci Animal Ind. *2*, 565.
Halpin, Holmes and Hart (1936). Poultry Sci., *15*, 99.
Evvard, Brown, Culbertson and Hammond (1926). Iowa Agr. Exper Res. Bull., *94*, 133.

CHAPTER

IX

Iron, Copper, and Nutritional Anemias

ALL THE higher forms of life iron is an integral constituent of
e, also called hematin, the prosthetic group of various complex
nents required in the transfer of oxygen. The most commonly
wn of these substances is hemoglobin, but myohemoglobin and
chrome are other important examples. It is estimated that the
ulating hemoglobin of a normal adult man contains about 2-2.5
of iron. Copper has not been demonstrated to occur in any
ific combinations in the higher forms of life but in recent years
as been shown that the element is required to promote the utili-
on of iron in hemoglobin formation, probably as a catalyst. Lower
nisms such as Mollusca and Arthropoda have a copper-containing
iratory pigment, hemocyanin, which acts as an oxygen carrier in
anner analogous to that of hemoglobin. Hemocyanin contains
t 0.34-0.35 per cent copper. It is significant that this figure is
tically identical with the value for iron in hemoglobin, about
per cent. Since these elements occupy integral roles in cellular
iration it is natural that nearly all reference to them in relation
utrition concerns anemia and blood regeneration.

Anemia may be regarded as due to loss or abnormal destruction
lood, or because of deficient blood formation. Anemias of nutri-
al origin are in the category of deficient blood formation, owing
n inadequate intake of structural components or activators in
atopoiesis, or deficient utilization of such nutrients. There is, of
se, no clear line of distinction between all the so-called nutritional
nias and those attributable to distinctly different causes such as
blood loss, acute blood destruction, and injury to blood-forming
ns by metastatic carcinoma, chemicals, infections, and physical
ts.

It is intended in this chapter to review some of the pertinen and opinions concerning the relationships of dietary iron an per to blood formation and anemia prevention, the factors af the utilization of these elements, their metabolism, and huma animal requirements for iron and copper. In addition, the re of diet to the etiology and treatment of pernicious anemia w briefly discussed.

Early Views Regarding the Role of Iron in Blood Formati Hirsch (1885) gives an interesting historical account of referen Ancient and Mediaeval writings to pallor or a "yellow tint" o skin as symptoms of illness and conjectures that these terms si chlorosis. According to Hirsch, Sennert in 1760, was the first t ploy this term, meaning, in the modern sense, a form of anemia common in young women and characterized by a marked redu in hemoglobin and a slight diminution of red corpuscles. Also, H states, there can be no doubt that chlorosis has been among the jects of medical observation at all times and that it is indepe of climate, soil, and race. He records that the "general anemi the tropics is principally the consequence of deficient food, or a poor in animal substances, and that malaria contributes to it. discourse is limited to chlorosis, general anemia of the tropics, "mountain sickness."

As stated by Robscheit-Robbins ('29), in an excellent revie the literature on blood regeneration, iron has been regarded a important factor in hematopoiesis since Menghini, in 1747, disco this element in blood. But it appears that even ancient civiliza held iron-containing waters in high esteem. Robscheit-Robbins p out that the main interest of iron studies between 1850 and centered on absorption and elimination of the element. But t studies resulted in scarcely any worthwhile contributions to the of iron in preventing anemia. Bunge (1889) is generally regarde the originator of the present view that iron deficiency is the cipal cause of anemia. Unfortunately he was led to believe that the iron contained in foods (organic iron) could be utilized in b formation. This view was shared for some time by Abderha (1900), Bunge's pupil. These two workers have the distinctio being the first to record that prolonged restriction of animals to diets causes anemia. Eventually Abderhalden, at the beginning of century, found that inorganic sources of iron are effective in b formation.

After the demonstration by many investigators that inorganic of iron could be absorbed and utilized for hemoglobin regenera the puzzling observation continued to be made that the admini

of iron compounds failed, in many instances, to bring about ef from anemia. Eventually it was realized that there are various es of anemia having origin in different causes. As early as 1843 dral (1843) first described the blood in "spontaneous" and "sec- ary" anemia. In the same decade Addison (1849) characterized nicious anemia—and initiated the study of endocrinology. Simple ochromic anemia was described by Faber in 1913. As distinguished n pernicious anemia, there are now recognized certain so-called ndary anemias. The most common cause of anemia is deficiency ron for the construction of hemoglobin. This is due to the lack ufficient available (utilizable) iron in the diet, or to conditions in alimentary tract which are inimical to its absorption. Anemias ch accompany hookworm infestation are due to chronic blood loss the inability of the body to restore the loss, often due in part nadequate intake of iron and other essential nutrients. The anemia nalaria is also due to blood destruction. Nephritis, rickets, scurvy, edema, malignancies, and several other conditions usually are mpanied by anemias. We will not attempt to classify them here. endeavor to learn the relationships between these apparently erent causes of anemia occasioned an enormous amount of research the accumulation of many detailed descriptions of the blood pic- in anemic individuals. The results of these various investigations among the great achievements in experimental physiology.

The Discovery of the Indispensability of Copper in Hemato-sis.—It has been shown by Bunge (l.c.) that milk is deficient in and that in the newborn young, with the exception of the guinea there are stores of iron in the liver and spleen sufficient to tide n over the suckling period. The guinea pig is so far developed rth that it is able to secure iron in the succulent vegetables which gins to eat within a short time after it is born; hence it does need a reserve supply of iron. Following Abderhalden's (l.c.) onstration that inorganic salts of iron cause hemoglobin forma- in animals restricted to a milk diet, the problem was not further stigated critically until Hart and his associates took it up in 1925 rt, Steenbock, Elvehjem, Waddell, and others, '25, '28).

n their first study they showed that rabbits suffered a marked ction in hemoglobin and red cells when limited to whole milk lemented with sodium citrate (added to soften curds formed in stomach). Ferric oxide added to this diet did not correct the nia. But ferric oxide together with fresh cabbage, or an alcoholic ct of desiccated cabbage, or of yellow corn meal, cured the nia although these extracts were free from iron. Chlorophyll, free iron, had a similar beneficial effect on hematopoiesis.

In later studies Hart and coworkers (1928) employed rats
these animals also developed anemia when restricted to the milk
The condition was not cured by the daily administration of 0.5
of iron as pure chloride, sulfate, citrate, acetate, or phosphate.
the anemic condition was quickly alleviated when the same d
of iron was administered in the form of the ash of dried beef
lettuce, yellow corn, or as acid extracts of these ashes. This inevi
led to the conclusion that their effectiveness was due to some
ganic element in addition to iron. The precipitate obtained by t
ment of the ash solution with hydrogen sulfide was considerably
potent than the original material. Eventually it was demonstrated
the active substance is copper, and that without it iron canno
utilized in hemoglobin formation.

Controversies Regarding the Significance of Copper.—Fol
ing this fundamental discovery various investigators became acti
interested and a controversy soon ensued over whether copper
be replaced by certain other elements in hematopoiesis. Titus, C
and Hughes ('28) asserted that manganese could replace copper
hematopoiesis but further work has failed to substantiate the cl
(Krause, '31). Myers, Beard and coworkers ('29) were the most
gressive proponents of the view that iron alone was sufficient to ind
hemoglobin regeneration under the experimental conditions emplo
by Hart and associates. They claimed, however, that the supplemen
tion of milk diets with manganese, nickel, germanium, and arse
could, like copper, increase the rate of hematopoiesis. Mitchell a
Miller ('29) also expressed the belief that a group of elements, rat
than copper alone, could function in hemoglobin building. Some cl
fication of the problem was furnished by Keil and Nelson ('31) w
found that pure ferric chloride alone promoted hematopoiesis wh
the milk employed was not carefully protected from metallic conta
nation. When the milk was collected in glass, ferric chloride did
cause improvement in the blood. After testing vanadium, titaniu
manganese, nickel, arsenic, germanium, zinc, chromium, cobalt,
and mercury, they concluded that copper is the only element wh
must be provided along with iron to cure or prevent anemia p
duced by Abderhalden's procedure, the restriction of animals to wh
milk diets. Others (Underhill, Orten and Lewis, '31; Cunningha
'31) have fully demonstrated that copper is the unique element nec
sary for iron utilization in hemoglobin production.

In retrospect it is not difficult to understand why the results
different investigators were in disagreement. As shown by Sheld
and Ramage ('32), copper was absent in only one out of five the
peutic preparations of iron examined by the spectrograph, and ma
ganese was a constant impurity. Also Davies ('33) found the natu

per content of milk to vary between 0.30 and 0.75 p.p.m. Obvi-
y, therefore, it was difficult for investigators to be certain that
i mineral supplements were pure and that the milk was com-
able in copper content. The entire subject has been reviewed by
ehjem ('35).

Cobalt Polycythemia.—Although cobalt apparently plays a signifi-
physiological role in nutrition (Chapt. XI) particularly in hema-
oiesis, there is no clear evidence regarding its function. The ad-
istration of only 0.5 mg. of this element as the chloride or sulfate
duces a polycythemic state in young rats (Orten, Underhill,
grage, and Lewis, '32). The effect was first noted by Waltner and
tner ('29). As shown by Orten ('36), cobalt administered to young
dult rats causes a reticulocytosis about the 4th day and a definite
cythemia by the end of 6 weeks. He believes that the polycythemic
e is due to an increase in the rate of hemoglobin and red cell
nation and not to diminished red cell destruction. This opinion
upported by the data of Barron and Barron ('36) on the basis
ed cell respiration studies. The latter workers believe that cobalt
resses the respiration of immature cells in the bone marrow, thus
ing them to be thrown into circulation. The result is an excess
irculating erythrocytes.

Goat's Milk Anemia.—The so-called "goat's milk anemia" has
n studied by several investigators. Rominger and associates ('33),
vell as others, claimed that it is not curable by iron nd copper,
itamin A and ascorbic acid. Since liver extract, but not gastric
osa, was said to be effective they postulated that the alleged goat's
anemia was due to a deficiency of Castle's "extrinsic" factor, a
othetical substance necessary in the prevention of pernicious
nia. But at present there is little credence in the view that this
n of anemia is essentially different from the simple type pro-
d by restriction of animals to cow's milk diets (Kohler, Elvehjem
Hart, '35; Beard and Boggess, '35; Orten and Smith, '36). Since
ler and associates have shown that the growth-promoting prop-
of goat's milk is low, unless the lactating animals have a good
It is possible that the confusion in this matter had its origin
e use of milk from poorly fed goats. The subject has been re-
ed by Orten and Smith.

ietary Factors Other than Iron and Copper in Hematopoiesis.
e illuminating researches of Whipple and Robscheit-Robbins
scheit-Robbins, l.c.) showed that the character of the diet, as
cts other factors than the iron content (exclusive of copper), is
traordinary significance in determining the capacity of the body
generate hemoglobin. Their method was based principally on
moval of measured amounts of red blood cells from experimental

dogs and observation of the effect of specific foods on the regene of hemoglobin. The routine experiments included the determi of plasma volume, hemoglobin, and erythrocyte and leucocyte on blood withdrawn by venipuncture. The following table sho influence of several foods on hemoglobin production:

HEMOGLOBIN PRODUCTION INFLUENCED BY DIET

Diet, Grams Daily	*Hemoglob Production week Feeding grams*
Bread, 400	3
Milk 450, Bread 400	3
Cream 100, Bread 400	10
Butter 100, Bread 350	15
Asparagus 200, Bread 300	9
Spinach 200, Bread 300	15
Raspberries 200, Bread 300	5
Raisins 200, Bread 300	25
Apricots 200, Bread 300	48
Eggs 150, Bread 300	45
Whole fish 250, Bread 300	13
Beef muscle 250, Bread 300	17
Pig muscle 250, Bread 300	30
Chicken gizzard 250, Bread 200	80
Kidney 250, Bread 300	70
Chicken liver 250, Bread 300	80
Beef liver 300, Bread 300	80
Beef liver 450	95

The precise basis for differences in the value of these foods not been determined. Obviously it was not entirely attributab differences in the iron content since raspberries have a higher value than apricots, yet the former were practically inert. The po of liver and kidney was regarded as due to the presence in parenchyma of a large amount of potential red cell and hemog building material other than iron. These data attracted much atte and owing to the high potency of liver, Minot and Castle beg investigate the efficacy of this food in the treatment of perni anemia. That it is remarkably effective was soon learned. A discu of the subject will be given subsequently.

It is of particular interest that Hogan and Ritchie ('34) observed an anemia in rats caused by the ingestion of deami casein. The evidence suggests that casein contains a chemical g which overcomes the toxic action of deaminized casein and w occurs in very small amounts, if at all, in gelatin and gliadin (C VI). As shown by Sebrell ('30), onions, either cooked or raw, given in quantities of 15 gm. or over, per kilogram of body we produce a severe anemia in dogs. Gruhzit's ('31) data suggest the effect is caused by certain disulfide compounds which occ

ons. It has not been demonstrated that anemia is caused in humans
he ingestion of onions.

Several investigators have observed anemia in animals on diets
arently deficient in one or more components of the vitamin
mplex. Miller and Rhoads ('32-33) claimed that dogs on a vitamin
deficient diet developed marked anemia although the diet con-
ed iron. This was not confirmed by Stucky and Brand ('32-33).
L. Day, Langston and Shukers ('35) have reported that severe
mia and leukopenia, with ulceration of the gums, occur in monkeys
icted to a diet of casein, whole ground wheat, polished rice, cod
oil, salt mixture, and oranges. Since the syndrome could be
ented by supplementation with dried brewer's yeast, the evidence
gests the involvement of some component of the vitamin B-complex
lood formation. Suzman and associates ('32), while studying the
t of vitamin A deficiency on the spinal cord, using dogs, employed
et consisting of 76.8 per cent of rolled oats supplemented with
r, lard, bone ash, a salt mixture, and calciferol (vitamin D).
mia developed on this diet, irrespective of whether the animals
e given vitamin A. Loss of weight, dermatitis, skin ulcers, and
ation in the concentration of blood lipids also occurred. The diet
deficient in some components of the vitamin B-complex. This
suggested as the cause of the anemia since iron was ineffective
elieving the condition. Various other evidences might be cited
he complexity of nutritional factors in blood formation.

Absorption of Iron.—Iron is almost entirely excreted by the in-
he. This fact makes it difficult to study iron absorption quantita-
y, since it is not now possible to differentiate between "ingoing"
"outgoing" iron. According to Witts ('36), iron is absorbed
ly by the duodenum, but the stomach and the whole of the small
tine may take part. Schmidt ('28) depleted mice of iron by
cting them during a period of 3-4 generations to a diet of milk
rice. The iron content of the intestine could not be made to
ly disappear, but was reduced to a very low minimum. There
no detectable stores of the element in the tissues. He then fed
compounds to these animals and made histological studies of
ntestines. It was found that iron is absorbed by the tips of the
of parts of the duodenum and appears in the underlying cells,
ently already combined with preformed cell granules. Absorption
place both through the portal circulation and the lymphatics, and
was carried thence to the liver and the mesenteric lymph glands.
iron which promptly appeared in the liver was diffusely dis-
d, but at this time there was none demonstrable in the spleen.
sis was not evident in the spleen until the hemoglobin content
blood had returned to approximately normal. From this it

appears that visible iron deposits in the spleen and liver rep degradation products of hemoglobin. Freshly absorbed iron, wh presumably stored in the liver, stains diffusely, or is present in co granules.

There is general agreement that iron, to be absorbable, mu soluble, ionizable, and ultrafilterable. Such forms are reduced salts soluble in acid solution, and the hydroxides of iron, whic readily changed to salts by acids. Hematin iron, and such co ions as ferro- and ferricyanide, do not pass through the intestinal Lintzel ('31) has shown that hematin iron is not liberated by prolonged peptic digestion.

Availability of Iron.—It has been thoroughly demonstrate various laboratories that inorganic salts of iron can be used a sole source of iron for the cure of iron-deficiency anemia in rats, chickens, and pigs (Elvehjem, '32). It is concluded therefore tha body's need for iron can be entirely satisfied by inorganic iron pounds. Since organic forms of iron, unless they are readily dige tend to lack the properties required for absorption, it is evident the inorganic forms are generally superior. Unfortunately there i generally satisfactory method of determining the form in which exists in foodstuffs. However, the method devised by Hill ('30) been of much use as a means of estimating chemically the approxi availability of iron in various materials. Hill's method is based on use of $\alpha\alpha'$-dipyridyl, a reagent which differentiates between ioniz and non-ionizable iron. Since there seems to be a fair correspond between the "biologically" available iron and the iron determine Hill's reagent, the chemical method has been used in various inv gations of iron in its relation to nutrition. Sherman, Elvehjem Hart ('34a) employed the reagent in an investigation of the avail iron in several foodstuffs. According to their findings the frac of the total which is available in wheat, oats, and yeast is 47, 57, 47 per cent respectively. Beef skeletal muscle has an availability o per cent, while that of oysters, spinach, alfalfa, and blood is 25 cent or less. These workers claim that hemoglobin regeneration of the above materials agreed with the values furnished by dipyridyl reagent. But the general reliability of $\alpha\alpha'$-dipyridyl been denied by a few workers. Rose and associates ('34) and and Bing ('36), employing biological assay methods, claim that wh for example, contains a much higher percentage of available iron t is indicated by means of this chemical procedure. As Borgen Elvehjem ('37) have shown with further evidence, the dipyr method, in general, does give a good indication of the availabilit iron in various substances. The content of available iron in foodst will be given subsequently.

Hill's reagent is extremely valuable in investigations of biological lems relating to iron. The compound combines with ferrous but ferric iron at pH 3.5 to 8.5, forming an intense red complex. ic iron can be determined after reduction, sodium hydrosulfite rally, being employed. It has been shown by Lintzel ('31, l.c.) ferric iron, but not ferrous iron, readily forms complexes with eins and sugars. This is important since ferric salts would tend ombine with mucus and undigested protein in the digestive tract, eby becoming unabsorbable. But in the alimentary tract the oxygen on is low and there are present many easily oxidizable substances, uation which would tend to reduce ferric to ferrous iron, in which it would become ionizable and absorbable. This view is sup- ed by the observation of Lintzel ('33) that anemia can be produced ats by feeding them dipyridyl while on a diet which normally ld maintain adequate hematopoiesis. If they had been able to rb ferric iron, anemia should not have occurred. However, this not suggest that ferrous iron should be taken in preference to ferric form. As we have indicated, ferric iron is readily reduced e ferrous state in the gastrointestinal tract.

erric iron evidently becomes available only when hydrochloric acid esent in the stomach since in achlorhydria anemia occurs. It ap- s that hydrochloric acid is important for the solution and reduction od iron in preparation for its absorption. In achlorhydria there be a failure to liberate iron from protein thus preventing absorp- of the iron. Organic acids in the food influence the solubility of and the amount of iron in solution in the stomach is greater than be accounted for on the basis of pH, owing to the presence of acid.

he Ca:P Ratio in Iron Utilization.—That other factors affecting olubility of iron in the gastrointestinal tract play important roles e utilization of iron is made evident by the studies of Orten, and Mendel ('36), Day and Stein ('38), and others. The former ed that when rats were fed a diet qualitatively complete but low organic salts, there occurred an increase in the number of ery- ytes per unit volume of blood and a simultaneous decrease in oncentration of hemoglobin. The diet was deficient in calcium o a lesser extent in sodium, potassium, magnesium, chloride, and Since the diet contained 18 per cent of the phosphoprotein, casein, hosphorus content was relatively high. Orten and associates were o prevent the abnormal blood picture by the provision of calcium nate. Since small amounts of ferric chloride were only partially ve they postulated that there exists some subtle relationship en calcium and the utilization of iron in blood formation. rnizing that excess of dietary iron causes a marked loss of phos-

phorus from the body as shown by Brock and Diamond Deobald and Elvehjem ('35), and others, Day and Stein believe an excess of phosphorus would cause the loss of iron, thus accou for the effects observed by Orten et al. They investigated the su and found that calcium does not have a unique function in her poiesis. When the low-mineral ration was supplemented with cal carbonate the results of Orten and associates were confirmed bu same amount of calcium, furnished as the diphosphate, was to without effect. Moreover, supplementation of the diet with gen amounts of ferric chloride, so as to remove most of the excess phorus, as iron phosphate, produced a normal blood picture the same amount of iron as ferric phosphate was without effect. addition to the diet of beryllium carbonate, which produce insoluble phosphate, likewise prevented abnormal hematopo Finally, by means of a basal ration adequate in all respects it possible to produce mild anemia and polycythemia by adding excess of phosphorus or by the reduction of calcium to a low l It was concluded, therefore, that neither calcium deficiency no extremely low Ca: P ratio are incompatible with normal hematopo if the diet contains some factor capable of preventing phosph from combination with iron. They stated, however, that calcium is only cation that is of practical value in this respect and that to as efficient utilization of iron attention should be given to the provi of an adequate Ca: P ratio. Thus it appears that the mechan whereby calcium "spares" iron has been demonstrated. Von We (1905) and Sherman (1907) first announced this relationship and shown clinically by Davidson and associates ('33), a normal bl picture can be maintained on a diet rich in calcium and moderat iron, but anemia occurs on the same iron intake if the calciu considerably reduced. It would seem that the clinical significanc this relationship should be further investigated.

Vitamins in Utilization of Iron.—Although, in general, ther no more similarity between vitamins as respects their physiolog roles than there is between minerals, for example, it is importan note that anemia accompanies deficiencies of vitamin A and asco acid. When the Ca: P ratio is unfavorable (low), as shown by Day Stein (l.c.), vitamin D aids in reducing the degree of anemia.

It seems probable that the anemia frequently observed in man degrees of vitamin A deficiency is related to the infectious states wh accompany vitamin A depletion. As pointed out by Josephs ('36) a review of anemia in infancy and childhood, infections cause man interference with hematopoiesis.

That anemia occurs in scurvy is well known, but it has b attributed by some to undernutrition and hemorrhage. Mettier

iates ('30) found in scorbutic patients (eight elderly men) that t rich in fruit, green vegetables, and fresh liver, all of which are in ascorbic acid, caused a prompt reticulocytosis and rapid re- ration of blood. Neither large doses of iron nor the substance it in pernicious anemia appeared to accomplish these results. ier and Chew ('31) concluded that the cytological changes in the marrow of scorbutic guinea pigs were indicative of retardation ythrocytosis. Pure ascorbic acid administration caused a marked topoietic response in a scorbutic man who was continued during xperiment on his deficient diet of bread, syrup, margarine, corned or smoked sausage, cheese, and tea made with sugar and canned (Dunlop and Scarborough, '35).

Metabolism of Iron.—From the available evidence, much of it of ous significance, it is possible to construct a general concept of metabolism. As pointed out previously, iron, to be absorbed, must luble and ionizable, and in the ferrous state. Unless the element e converted into this form before passing into the lower part of mall intestines, it is excreted. Iron in hematin, the pigment of globin, is not liberated by ordinary digestive processes and is fore not absorbed. In general, other natural organic forms of iron e similarly. According to Hill (l.c.) all of the element in egg is in inorganic form, probably as ferric hydroxide, and is there- potentially absorbable.

he absorbed iron appears to be taken up by the liver but the of that organ in the transformation of iron into respiratory ents is uncertain. Likewise, there is no conclusive evidence con- ng the function of the spleen in iron metabolism. However, it is r generally believed that this organ is important, but not indis- ble, in the degradation of hemoglobin from damaged red cells. extent of the body's ability to utilize iron from catabolized globin does not seem to have been thoroughly determined. As n by Stearns and McKinley ('37) the infant is able to utilize of the iron released during the period of physiological hemo- n destruction. The mechanism whereby iron is introduced into igment of hemoglobin is not known. That the bone marrow is te of erythrocyte formation is thoroughly demonstrated.

ahn and Whipple ('36) exhausted the iron reserves of dogs by roduction of prolonged anemia due to repeated bleedings. They performed viviperfusions to free the organs and tissues from . Following this the "parenchyma" iron (functional) was de- ned. In short feeding experiments of 1 to 2 days the iron bed was all used for the production of hemoglobin, and no iron accumulated in the liver. With longer feeding periods the storage small. The conspicuous feature was the rapid appearance of

ingested iron in hemoglobin. Viviperfusion carried out 24 hours the intravenous administration of iron showed that 55 to 70 pe of the iron was stored in the liver and spleen.

The non-hemoglobin parenchymal iron of various blood-free o was relatively constant. Average values per 100 gm. of tissue 1-2 mg. in liver, kidney and pancreas; 3 mg. in lung; 5-6 m spleen; and 10 mg. in red marrow. Striated muscle averaged 3.1 of which 1.6 mg. was present as myohemoglobin and 1.5 m parenchymal iron. These figures for muscle are constant and d increase when iron is given intravenously or decrease when an is severe and prolonged. In view of this it is suggested that hemog formation is subservient to the needs for non-hemoglobin iron. is supported by Josephs' ('32) observation that if a diet of mi continued beyond the normal nursing period, all the iron is use maintain the "functional iron" at a constant, though minimal, l

According to Whipple and Robscheit-Robbins ('36), in stan anemic dogs (vide supra) an optimum dose of iron (40 mg. per for 14 days) will give a net output of about 55 gm. hemoglobin. utilization is therefore about 35 per cent. The iron was utilized equal facility when given orally whether in the ferrous or ferric s

The Content of Available Iron in Foodstuffs.—As we h pointed out previously, only ionizable iron reacts with $\alpha\alpha'$-dipyri Since this form of iron is biologically available while other forms not, the dipyridyl method has been employed to estimate the availa iron in foodstuffs. The following table, prepared from data given Sherman, Elvehjem and Hart ('34a, l.c.), indicates the marked ferences between biological materials as respects available iron comparison to total iron:

AVAILABLE IRON ESTIMATED BY DIPYRIDYL METHOD

(Results expressed in mg. per 100 gm. of sample)

MATERIAL	TOTAL Fe	AVAILABLE Fe	PER CE AVAILAB
Pork liver (dry)	65.22	43.48	67
Beef liver (dry)	26.08	18.26	70
Pork heart muscle (dry)	15.31	13.26	87
Beef heart muscle (dry)	24.00	16.80	78
Beef skeletal muscle (dry)	14.08	7.04	50
Oysters (dry)	31.92	7.98	22
Soy beans (roasted)	10.00	6.00	60
Soy beans (non-roasted)	9.04	7.29	80
Spinach (dry)	56.23	10.94	20
Alfalfa	12.78	3.52	27
Blood (rats)	37.50	4.00	11

Other useful data are those of Shackleton and McCance ('36) who determined the ionizable iron in a large number of English stuffs. The following table was prepared from their data:

AVAILABLE IRON ESTIMATED BY DIPYRIDYL METHOD

(Results expressed in mg. per 100 gm. of sample)

MATERIAL	TOTAL Fe	AVAILABLE Fe AS PER CENT OF TOTAL Fe	MATERIAL	TOTAL Fe	AVAILABLE Fe AS PER CENT OF TOTAL Fe
age, raw	0.98	72	Grape, dry (raisins)	3.80	97
t	0.56	100	Grape, black, raw	0.27	85
t, cooked	0.41	98	Grapefruit, raw	0.11	92
, raw	0.14	100	Lemon juice	0.07	80
ce, raw	0.80	63	Orange juice	0.20	85
, raw	0.40	100	Peach, fresh, raw	0.39	100
y, raw	10.00	50	Pear, raw	0.21	100
plit, cooked	1.84	71	Pineapple, raw	0.22	91
, cooked	0.62	97	Plum, dry (prunes), raw	3.20	72
h, cooked	4.15	57	Tomato, raw	0.37	64
raw	0.24	100	Peanut	1.19	100
t, dry, raw	4.08	98	Molasses, black	9.17	100
t, fresh, raw	0.37	95	Salmon, canned	0.89	94
a, raw	0.47	100	Sardines, canned	3.44	65
erry, raw	0.95	40	Beef, roast	5.20	19
, raw	0.48	100	Mutton, roast	5.10	24
erry, raw	0.70	70	Ham, cooked	4.45	15
dry	1.71	82	Veal, roast	1.35	55
y, raw	4.17	96	Egg, raw	2.50	100

will be noted that the iron of eggs is entirely available. Egg in is almost free of iron. As shown by Elvehjem and Hart (l.c.) olks of hens' eggs contain about 0.0143 per cent iron. The value not be increased by feeding large amounts of iron, or of iron opper. In the yolk there is a Cu:Fe ratio of about 1:19. In rats anemic by restriction to a milk diet the iron of egg yolk is poorly d unless copper is furnished (Sherman, Elvehjem and Hart When anemic rats received 0.3 mg. of iron daily in the form ric chloride, hemoglobin regeneration was normal; when this nt was provided as egg yolk, regeneration was poor. Supplemention of the egg yolk with 0.05 mg. of copper, as copper sulfate, ot as effective as 1 mg. daily of copper. As discussed in the on availability of copper, it is apparent that some of the copper nverted into the sulfide, by hydrogen sulfide from the egg yolk, ecoming unavailable.

Ferric chloride, ferric pyrophosphate, and ferric hypophosph 100 per cent available. The value for ferric glutamate is about cent. Elvehjem, Hart and Sherman ('33) have pointed out that pyrophosphate possesses several desirable qualities as a dietar supplement. It is quite soluble in water and not astringent most soluble salts of iron.

The distinction between total and available iron should be tained clearly in the subsequent discussions of iron requirement

Metabolism of Copper.—Prior to the discovery that cop essential for iron utilization, Bodansky's ('21) few analyses of h brain had indicated that fetal brains contain more copper than of adults. This suggestion of intrauterine storage was further ind by the studies of McHargue ('25) who found that the liver newborn calf contained 8 times as much copper as did that of grown animal. Since milk is deficient in copper it is obvious intrauterine storage of this element is a necessary provision of n Various studies in recent years have indicated that the new mammal is furnished with enough copper in the liver and other t to meet its needs during a large part of the normal suckling p

In order to develop some understanding of the role of copp hematopoiesis it is necessary to learn something of its metabo Lindow, Elvehjem and Peterson ('29) found that the absolute an of copper in the body of a normal rat increased from 0.0108 m birth to 0.4422 mg. at 210 to 240 days. This level could not be r by subjecting the mother to a high copper intake either before or parturition. The observation confirmed that of Elvehjem, Steen and Hart ('29) that diet does not appreciably affect the copper co of milk. But copper added to the diet was readily stored. Rats 85 days old on a normal stock ration with added copper, cont twice as much of the element as similar animals receiving the u plemented diet. Copper feeding increased the copper content o skeleton, kidney, spleen, and liver, 1.6, 2, 5, and 20 times, respect About 98 per cent of the excreted copper was in the feces when co was added to the ration. With the unsupplemented ration only 70 per cent was in the feces. Since practically all of the excreted is in the feces it is indicated that a difference exists in the partitio iron and copper.

Some indication of the distribution of copper in adult rat tissu afforded by the data of Lindow, Peterson and Steenbock ('29, given on page 225.

It is apparent that of the tissues examined only liver, kidney, spleen are important as storage organs. But, as shown by Schu Elvehjem and Hart ('36), young rats restricted to a milk diet ficient in Cu, as well as Fe, Mn, and perhaps other essentials) be

Tissue	Mg. of Cu per Kg. Dry Tissue		Tissue	Mg. of Cu per Kg. Dry Tissue	
	(*a*)	(*b*)		(*a*)	(*b*)
e	2.50	4.25	Lung	6.21	10.67
n	9.14	11.16	Muscle	2.00	2.10
rt	9.92	12.25	Skin	3.57	4.00
ney	12.41	27.20	Spleen	3.41	17.47
r	11.43	213.32	Testicle	8.65	10.00

a) = Stock ration alone; (b) = Stock ration plus added Cu for 46 days.

kedly depleted of bodily copper stores. In spite of this the feeding opper and iron for 7 days caused a retention of only 5 per cent of copper, although maximum hemoglobin formation occurred.

The relationship between copper in tissues and age is indicated by ningham's (l.c.) data in which the copper content is expressed as per kg. of dry substances:

	Liver	Heart	Lungs	Spleen	Kidney	Pancreas
t bovine . . .	77.0	15.6	5.3	2.9	19.7	3.8
newly born . .	470.0	14.8	4.9	4.8	15.7	5.5

According to Sheldon and Ramage's ('31) spectrographic analyses, per occurs in all tissues. Chou and Adolph ('35) state that the age adult body probably contains 100 to 150 mg. of copper.

Role of Copper in Hematopoiesis.—It is significant that rapid oglobin formation occurs when copper and iron are given to nals, depleted of bodily copper stores, although only a small unt of copper is retained (Schultze, Elvehjem and Hart, l.c.). ltze and associates were impressed by the observation that blood the only tissue studied in which a large accumulation of copper rred when copper was fed to an animal previously depleted of element. This suggested that the hematopoietic action of copper rely catalytic, therefore requiring but extremely small amounts in ctual process of hemoglobin formation. Schultze et al., who have ed with young swine, believe that rapid, continued hematopoiesis ot occur unless the content of blood copper is maintained above nimum level. This level is regarded as about 20 micrograms per c. of pigs' blood. It is perhaps significant, in view of this, that s and coworkers ('37) have noted a tendency towards the ac- ulation of copper in blood of patients with pernicious anemia,

while the iron content is low. As shown by Elvehjem and She ('32), pure iron added to the milk diet of anemic rats is dep in the liver and spleen to some extent, at least, and hemog production does not occur. But when the iron supplement placed by copper, rapid blood formation results, causing a r tion in liver iron to a level slightly less than that in severely an rats.

Although the various types of evidence suggest that copper in the introduction of inorganic iron into the organic complexes w form hemoglobin, there is no information on the actual mechanis the process. Elvehjem ('35, l.c.) in a review of the biological nificance of copper, states that "It is entirely possible that copp concerned with the normal functioning of general body activities, that the body's inability to form hemoglobin (in Cu deficie depends upon changes in these activities." Cohen and Elvehjem believed that copper might act through certain respiratory enzy Cytochrome was investigated since it is composed of three h ochromogens, *a, b,* and *c*. In rats depleted of iron and copper restriction to a milk diet the *a* component was absent or much red in heart and liver tissue, but only slight reduction occurred in th and *c* components. When copper alone or iron and copper were ad to the milk, the *a* component was greatly increased. It was necessar feed both copper and iron to increase the *b* and *c* components. S iron alone was not effective in restoring the *a* cytochrome, w copper was effective, it was indicated that copper has a catalytic in the formation of hemochromogens other than that required hemoglobin. These findings are in agreement with Elvehjem's (observations concerning the effect of copper on cytochrome produc in yeast. Since copper is extremely active in the oxidation of asco acid (Mawson, '35) and sulfhydryl compounds, in addition to ind tions of involvement in carbohydrate metabolism (Keil and Nel '34), there is reason to believe that it does play other roles in the b aside from hemoglobin production.

Copper Deficiency and Depigmentation.—As early as 1931 H and Nelson ('31, l.c.) noted that dark coated rats made anemic restriction to a milk diet underwent marked changes in the mentation of hair. Black hair became a silvery gray. Since i supplementation of the diet did not restore the original color, whe iron and copper together were effective, it was indicated that cop has a role in pigmentation. This effect was noted by Cunningh (l.c.) who found that the copper content of skin from black rats rabbits tended to be higher than that of albinos. He suggested t copper might serve as a catalyst in the formation of melanin pigme and referred to the possibility that copper in the ink sac of the octo

y be related to the formation of melanin in the ink. Gorter ('35) o confirmed this finding and found that of various metals tested y copper was effective. He claims that the dosage must be much her than for the cure of anemia. The relation of these findings to estions of pigmentation in skin and hair of humans has not been ermined.

Biological Availability of Copper.—Scarcely anything is known ncerning the forms in which copper exists in plant and animal ues. At present only four organic forms of copper are known initely to exist in nature. These are (a) turacin, a uroporphyrin npound which occurs as a highly colored copper pigment in the thers of a South African bird, Turaco; (b) hemocyanin; (c) polyenol oxidase; and (d) a compound recently isolated from ox blood Mann and Keilin ('38) who have proposed that it be called mocuprein. In the latter 3 compounds copper is united to protein, arently in specific combination. It is reasonable to suppose that he compounds of copper are practically unavailable to the body ile others are utilized with considerable efficiency. As shown by ultze, Elvehjem and Hart ('36), copper of wheat germ, alfalfa, wers' yeast, pork heart, pork liver, cystine cuprous mercaptide, per aspartate, copper citrate, copper nucleinate, and copper pyrosphate is utilized efficiently by severely anemic rats. Copper porrin and copper sulfide were poor sources of copper. It is possible, refore, that any foods, such as egg yolk, which release considerable ounts of hydrogen sulfide in the digestive tract, might cause the mation of insoluble copper sulfide, thus interfering with copper ization. This possibility should not discredit eggs or similar foods his respect, since the dietary of man apparently contains sufficient per to meet these possible contingencies.

The Content of Copper in Foodstuffs.—Lindow, Elvehjem and erson (l.c.) have reported the copper content of many animal and etable products, and give references to earlier analyses. They list foods containing less than 1 mg. per kg. of fresh material the owing: apples, beet greens (tops), cabbage, cantaloupe, carrots, ry, sweet corn, cranberries, cucumbers, gooseberries, grapes, grapet, kumquats, lemons, lettuce, muskmelon, onions, oranges, pinele, pumpkin, rhubarb, squash, strawberries, tangerines, tomatoes, nips, watercress, and watermelon.

Foods rich in copper, according to the analyses of these investiors, are on the fresh basis: liver (adult beef), 21.5; calf liver, 44.1; zil nuts, 14.8; butternuts, 11.7; chocolate (bitter), 26.7; cocoa, 33.4; asses, 19.3; mushrooms, 17.9; pecans, 13.6; walnuts, 10.0; wheat n, 11.7; and wheat germ, 12.7 mg. per kilogram, respectively. They e listed the average, minimum and maximum content of copper,

iron, and manganese in different classes of foods as shown following table:

DEGREE OF VARIATION IN IRON, MANGANESE, AND COPPER CONTE DIFFERENT CLASSES OF FOOD MATERIALS

CLASS	NO. OF SAMPLES	AVERAGE	MINIMUM	MAXIM
		IRON IN FRESH MATERIAL		
		mg. per kg.	*mg. per kg.*	*mg. per*
Fresh fruits . . .	23	6.6	2.3 Watermelon	22.8 Grapes
Nuts	12	41.0	21.4 Walnut	79.2 Pistachi
Roots and tubers .	14	11.0	3.0 Onion	23.6 Beets
Vegetables, leafy .	7	69.0	3.4 Cabbage	192.1 Parsley
		MANGANESE IN FRESH MATERIAL		
Fresh fruits . . .	13	4.0	0.2 Watermelon	22.9 Blueberr
Nuts	3	13.3	6.3 Pistachio	18.0 Walnut
Roots and tubers .	7	3.2	0.5 Onion	13.5 Beets
Vegetables, leafy .	8	6.6	0.8 Cabbage	12.6 Beet gr tops
		COPPER IN FRESH MATERIAL		
Fresh fruits . . .	27	1.0	0.2 Strawberries	3.4 Olives
Nuts	10	11.6	6.0 Chestnuts	14.3 Hickory
Roots and tubers .	11	1.4	0.8 Carrots	2.7 Oyster pl
Vegetables, leafy .	14	1.2	0.4 Watercress	3.1 Artichok

The content of copper in many foods will be found highly var since the widespread use of copper-containing sprays on fruits vegetables adds much copper to these products. Elvehjem and ('29) raised the copper content of lettuce 148 per cent by addin lbs. of copper sulfate per acre of the soil on which it was grown

Iron and Copper Requirements of Infants and Children. birth normal infants contain considerable stores of iron and co largely in the liver. Premature infants contain much less iron copper indicating that storage occurs late in *in utero* develop Also, the blood supply of infants at birth is larger than necessar the first few weeks of life since there are no placental tissues to r tain after birth. Consequently a "physiologic" destruction of b occurs during the first period of infancy. In this period, accordi

tearns and Stinger ('37), about 75 mg. of iron are excreted above hat ingested. They have estimated that to provide the iron necessary uring the remainder of the first year of life the infant would need retain 0.75 to 1.0 mg. daily after 2 months of age. Since milk is oor in iron as well as copper it is necessary to have other sources of ese nutrients early in life. The normal bodily stores alone are not le to provide for adequate hematopoiesis throughout the entire ursing period.

Various procedures have been employed to estimate the normal on and copper requirements of infants and children. Among these e metabolic balance method has had a prominent place but, as we ve pointed out elsewhere (Chapt. X), the method is deficient from e standpoint of both theoretical and practical considerations. How-er, it is not without some definite value. By means of this procedure is possible to determine the approximate iron balance in a few bjects over a limited period of time and deduce from the data some cts concerning iron and copper metabolism. Rose and associates ('30) udied a 31 months-old child and observed a negative iron balance ith a daily ingestion of 0.23 mg. of iron per kilogram. Leichsenring d Flor ('32) found, in 4 healthy children between 3 and 6 years, tentions of 0.07 and 0.18 mg. per kilogram at daily ingestion levels 0.19 and 0.36 mg., respectively. They estimated the daily minimum quirement to be 0.32 mg. per kilogram, and suggested that a 50 per nt margin of safety should be provided. It would seem that such ta should be accepted with caution, in the light of variability in the ailability of various forms of iron, and of the inability of the body utilize available iron unless a certain, at present unknown, minimum mount of utilizable copper is also present.

The practical question is: How much iron and copper of a given ailability must an infant or child ingest regularly to maintain normal matopoiesis? The amount excreted is of secondary importance in termining requirement. According to Elvehjem, Siemers and Men-nhall ('35), the average hemoglobin value for average infants and ildren between 6 months and 2½ years of age was between 11 and gm. per 100 cc. of blood. When 25 mg. of iron and 1 mg. of copper ferric pyrophosphate and copper sulfate, respectively, were used to pplement the regular diet of infants, the hemoglobin was raised m 9-11 gm. to 12-13.5 gm. per 100 cc. of blood. The use of 12.5 mg. iron and 0.5 mg. of copper did not give as uniform or consistent sults as the larger doses of these nutrients. The importance of copper fully demonstrated since Josephs ('31), Elvehjem, Duckles and endenhall ('37), and others, have shown that the response of anemic fants to iron is enhanced by copper administration. There is no finite information concerning the copper needs. As shown by

Daniels and Wright ('34) in metabolic balance studies of chi 4 to 6 years old, the average daily copper retention was 0.026 mg kilogram when the average ingestion was 0.086 mg. per kilog Higher retentions were found when 0.090 to 0.093 mg. per kilo were provided by the diet; hence, for safety they set the require of this age group at 0.100 mg. per kilogram. Hodges and Pete ('31), on the basis of their numerous estimations of copper in f have calculated the daily copper intake to vary from 0.8 mg. f child of 3-4 years to 4.81 mg. for an adult. On this basis the co intake of children scarcely exceeds the requirement suggested Daniels and Wright. The limitations of the metabolic balance me have been indicated.

Mackey ('31) and Josephs ('36, l.c.) have adequately reviewed subject of nutritional anemias in infancy and early childhood. T reports reveal the high incidence of such anemic conditions where dietaries are low in iron and copper and also ascorbic acid. T emphasize the importance of supplementing milk with iron copper.

The iron and copper needs of older children, especially adolesce are relatively unknown. Various data show that the average he globin and erythrocyte levels in adolescence tend to be definitely lo than in adulthood but higher than in young children. As shown Osgood and Baker ('35), there is no appreciable sex difference in prepuberal period. This is significant since in adults men genera tend to have higher values than women (Wintrobe, '30). An expla tion of this fact cannot be given yet, but it appears to be related differences in dietary practices, menstruation with the consequent l of iron, and physical development. It would seem that the iron a copper requirements in adolescence are definitely higher than adulthood but precise values or standards cannot be stated.

Iron and Copper Requirements of Adults.—In an investigati of the poorest classes of the population in Aberdeen and the nor east of Scotland, Davidson, Fullerton and Campbell ('35) found t the total daily iron intake was about 10 mg. per person and an inta of only 5 to 8 mg. per day was not an uncommon finding. Thirty-f hundred individuals of both sexes and at all ages were studied. the basis of their reasonable standards anemia was found in 41 p cent of infants under 2 years, 32 per cent of pre-school children, per cent of school children, 16 per cent of adolescent women, and per cent of adult women. It was generally absent in adolescent a adult males.

An indication of a satisfactory method of estimating the iron quirements is afforded by the data of Widdowson and McCance ('3 who evaluated the total and "available" iron intakes of 63 men a

omen of the English middle class living on freely chosen diets average intake for men was 16.8 mg., but 37 per cent of the men ingesting less than 15 mg. per day. The amount of available iron nen was 10.8 mg. per day. Thirty per cent of the women were ving less than 10 mg. daily and the average value for available was only 7.9 mg. The hemoglobin values, obtained on about alf of the subjects, indicated normal levels on the basis of current lards. But the ingestion of 100 mg. of iron daily as ferrous sulfate erric ammonium citrate increased the hemoglobin values for en by 4 to 17 per cent. This supplement had scarcely any effect en. The studies indicate, therefore, that the level of ingestion in was approximately adequate, but inadequate in the women.

rr ('35) found that men maintained normal hematopoiesis when otal iron intake ranged from 5.3 to 23.2 mg. daily. But women ting as low as 6 mg. daily tended to be anemic. Also, Farrar Goldhamer ('35) have reported that a male student 26 years old tained a normal blood picture throughout an investigation period 6 days, although the total daily iron intake was approximately . Moreover, 2 other young men had normal levels of hemoglobin rythrocytes, although the total daily intake was only 7.1 and 7.8 respectively. The three subjects remained in positive iron balance. mportant to note, however, that it takes more than three subjects ablish a dietary standard. Also, each of the individuals reported were ingesting at least 1 quart of milk daily. We have already ssed the significance of adequate calcium and the importance of a:P ratio in iron utilization.

veral years ago Sherman formulated a standard of 15 mg. of iron) for normal adults. Although it is possible that normal topoiesis can be maintained on levels of ingestion considerably this, especially when the other factors are favorable for utiliza- t would seem that a safe practice would be to follow Sherman's rd. If much of the iron is furnished by foods containing a low ntage of available iron, the level should be raised.

thing definite is known concerning the copper requirements in ood. Chou and Adolph (l.c.) believe that the copper intake be about 2 mg. daily. Since the copper content of most common uffs ranges from 2 to 25 mg. per kilogram of dry material, it is le that the average mixed diet supplies adequate amounts of ineral.

n Requirement in Relation to Menstruation.—Much has been concerning the effects of menstruation on the content of hemo- in blood, the general assumption being that this physiologic places a burden on the body owing to the iron losses. But as by Leverton and Roberts ('37) "the low hemoglobin values

which are accepted as normal for women because of the drai to menstruation may be a direct reflection of the use of diets habi low in iron rather than due to the small periodic loss in the me In four normal young women who were studied the average strual losses of iron were 14.26, 22.84, 11.13, and 13.80 mg. respec The losses were relatively constant from period to period for the subject. The values reported by Gillett ('18) and by Ohlson and ('35) are somewhat higher. The former observed a loss of 34 to 4 of iron per menstruation and the values given by the latter we to 42 mg. On the basis of Leverton and Roberts' data it is nece for a sexually mature woman to absorb and utilize approximate to 20 mg. of iron during each menstrual period, in addition to required to meet the other requirements which, presumably, are si to those of men. This means, however, that the diet must fu more than the given amount of iron to meet this particular nee addition to other requirements, since there is not 100 per cent effici in the utilization of dietary iron even though it might be in a which permits absorption.

In the group of young women studied by Leverton and Ro the average daily intakes of iron for the four subjects were 13.61, 1 10.03, and 11.71 mg. Presumably most of the iron was available s the foodstuffs used were relatively low in organic iron. On regimen the hemoglobin values increased from 12.95, 12.54, 11.62, 13.06 gm. per 100 cc. for the first menstrual cycle of the four subj to 14, 13.92, 13, and 14.3 gm. for the last cycle studied (Leverton Roberts, '36). These increases in hemoglobin, in spite of the intakes that approached the minimum of safety, are significant. T suggest that the previous dietary was poorer in available iron t the experimental regimen which was planned to represent a w chosen mixed dietary with no emphasis on foods rich in avail iron. On the basis of these studies it is possible to conclude that m struation does cause a significant increase in the requirement available dietary iron, but this physiologic function does not consti a large burden, probably no larger than a severe nosebleed. Also illustrates the tendency of ordinary dietaries to provide less t enough iron to permit maximal hemoglobin production in sexu mature women.

Iron and Copper Requirements of Pregnant and Lactat Women.—The iron and copper requirements are definitely higher pregnancy and lactation. It is generally observed that in pregnancy anemia occurs which, in women who have had no apparent def in diet or gastric function, tends to be abruptly alleviated withi few days after parturition. As stated by Strauss ('34), there is a ste decline in both hemoglobin and erythrocytes from early in the pr

y until the end of the sixth month, after which there may be no
er change, or a slight increase might occur. There are various
s of anemia in pregnancy, among which are infections and an
equate intake of available iron, but according to Strauss the
lled "physiologic anemia" is mere hydremia.

lterations in blood volume, with a consequent reduction in the
entration of red cells and pigment, do occur as shown by various
ers including Beard and Myers ('33) and Van Donk, Feldman
Steenbock ('34) using rats, and Schultz ('33) and Smallwood
who studied human subjects. Adair, Dieckmann and Grant
state that in their pregnant subjects the mean hemoglobin con-
ation was 11.56 gm. per 100 cc. of blood and there were 3.77
on erythrocytes per cmm. Their minimum standards for preg-
y were stated to be 10 gm. per cent of hemoglobin and 3.36
on erythrocytes per cmm. On the basis of this standard 11.6 per
of the pregnant patients were anemic, but on the basis of stand-
for non-pregnant adults 63.2 per cent were anemic. These findings
be compared with those of Davidson, Fullerton and Campbell
. They used the Haldane hemoglobinometer (100 per cent = 13.8
Hb. per 100 cc. of blood) and the Price-Jones standard of 98 = 10
ent. In 819 pregnant women, aged 15 to 44 years, the average
globin was 78.1 per cent (10.78 gm. Hb. per 100 cc. blood). In
arous non-pregnant women, aged 15 to 44 years, the average
globin was 81.3 per cent (11.22 gm. Hb. per 100 cc. blood), and
one-half of the values were below 85 per cent. The hemoglobin
d to be lower in the older individuals. Values for the pregnant
n were below those of non-pregnant women of the same age
. The data suggest that there is a progressive anemia throughout
productive years which is increased by pregnancy.

ard and Myers (l.c.) have suggested that the physiologic anemia
gnancy is somewhat analogous to the anemia occurring during
growth. Whether this is a valid analogy has not been fully
d, but the evidence does not suggest that hydremia is as pro-
ed in growing persons as in pregnant individuals. The literature
ysiologic anemia has been reviewed by Garry and Stiven ('36).
ce large amounts of iron and copper are stored by the develop-
us, it is apparent that the diet must supply generous amounts
se nutrients if marked hypochromic anemia is to be avoided.
an and Strauss ('36) conducted an important study in which it
emonstrated that the blood of apparently normal pregnant
n can be considerably improved by supplementation with iron.
he patients in the antepartum clinic were given coated tablets
ing ferrous sulfate and the other half were given tablets similar
arance but containing lactose and no iron. The difference was

not divulged to any of the patients. The treated group receiv average of 0.5 gm. of ferrous sulfate daily for an average of 162 Of the 100 women who received no iron, 24 had less than 70 pe hemoglobin (100 per cent by this standard is normal) post pa Of the 100 women who received iron none had less than 70 pe hemoglobin post partum. The significance of these data is app

The extent of the demand for iron by the human fetus is clear by Coons and associates ('35) who summarized the results published analyses. The average iron content of the fetus at was 395 mg., and the maximum was 937 mg. It was calculated tha content of blood iron would be between 238 and 437 mg. if the volume of a 7.5 lb. infant should be 14.7 per cent of the body w and the blood hemoglobin were 103 and 189 per cent. They estim a possible iron store of 160 mg., exclusive of that in the blood this basis the iron contribution of the mother during pregn would be 400 to 600 mg. They suggest that the diet should pro for the absorption and utilization of 500 mg. of additional iro the pregnant woman. Coons and associates found a daily iron in of 15 mg. just sufficient to maintain a positive iron balance in preg women, but Toverud ('35) found negative balances when onl mg. of iron were ingested daily. According to Macy and Huns ('34), the desirable intake is 20 mg. daily during pregnancy. refers to available iron.

Scarcely anything is definitely known about the iron and co requirements of lactating women. It is certain, however, that they larger than in non-lactating mothers since the milk contains some and copper, although the amount is relatively low.

Anemia in Domestic Animals.—Anemia in suckling pigs has b reported to occur in Scotland, Sweden, Ontario, and elsewhere. It been the source of great economic loss due to unthriftiness and ex sive mortality. The condition appears to have been first described McGowan and Crichton ('23). They found, as have others, access to a supply of iron, e. g., ferric oxide, caused an immed return of appetite and improvement in condition, the hemoglo rising almost to normal in about three weeks. Since the discovery an anemia resulting from cobalt deficiency, and of the necessity copper for iron utilization, it is not certain whether the anemia young swine is, in all cases, due specifically to iron deficiency o other causes. The sources of iron used to treat anemic swine w undoubtedly impure. The details of the blood picture in this condit attributed to iron deficiency, have not been described. Davidson Leitch ('34) have reviewed the literature on nutritional anemias various domestic animals and man.

The type of anemia in cattle and sheep known as "bush sickne

ast disease," and "pining," will be shown subsequently to be due deficiency primarily of cobalt (Chapt. XI). This disease is apparly identical with the "salt sick" of Florida. In certain instances the er condition has been reported to be cured by the provision of iron copper, but not by iron alone. This suggests that in some instances, he reports of this disease, deficiency of copper may have been the se, except when the efficacy of cobalt was established. It would n that anemia in animals is in most instances referable to deficiency ron, copper, or cobalt.

Anemia in poultry appears to be very uncommon under farm ditions. It may be induced by a milk diet as has been shown by t and associates ('29-30). The hemoglobin level, which varies from 19 gm. per 100 cc. of blood, tends to fall during periods of high production (Cook and Harmon, '33). Graham and Smith ('29) rted the results of incubation tests of more than 40,000 hen's eggs n the same genetic stock, which showed consistently low hatchity during February, March, and April, whereas the hatchability markedly during May, June, and July. This change corresponded range freedom and accompanying improved quality of the diet ss, sunshine, insects, etc.). They attributed the results particularly deficiency of the fat-soluble vitamins, but it is possible that a ciency of copper or iron was at fault. Studies of similar diets by Farlane and associates ('30) showed that the iron content of the was little, if any, influenced by the food of the hen, but the copper ent was greatly affected by the diet. Many of the chicks, or ryos, from eggs of low hatchability were anemic, especially when age or meat meal were fed to the hens. These studies are mened because they suggest need for investigations concerning the ion of iron and copper to hatchability of eggs.

ernicious Anemia.—Physicians long ago recognized that certain of anemia were accompanied by parasites, chronic hemorrhage, ntery, malaria, syphilis, or bacterial toxins, etc., and that recovery kly followed when the cause was removed. The anemias were, fore, "caused" or "secondary." Iron tonics, or chalybeate waters found useful in promoting prompt recovery. Addison's descripof the disease since known by his name, in 1849, has been already ioned. The symptoms first recognized were loss of weight, pallor, ude, anorexia, soreness of the tongue, gastric disturbances, and bness of the extremities. Since there was no known successful ment, the syndrome became known as "pernicious anemia." In Ehrlich introduced the technic of staining blood smears by ne dyes. This brought about rapid increase in knowledge of the acteristic details of the blood picture in different types of anemias. 1890 there was a wave of enthusiasm for analysis of stomach

contents, and as data accumulated on gastric analysis it emerged there was a relationship between lack of free hydrochloric acid in stomach secretion and pernicious anemia. Patients with this disease infrequently showed lack of peptic activity. Unlike the secon anemias, pernicious anemia was apparently not due to any su imposed disorder; hence it became known as "primary" anemia. S investigators continued to believe that pernicious anemia was essentially different from other anemias, and that the cause shoul discoverable. The researches which finally led to the present un standing of the etiology of pernicious anemia constitute one of most remarkable achievements in the history of nutriti investigations.

Red blood cells are produced in the bone marrow, and in normal individual they are passed into the blood stream where t are buffeted about during 30 to 40 days, by which time they bec considerably damaged and are disposed of as waste material. A given time the number of red cells in the blood represents the bala between formation and destruction. In pernicious anemia the b marrow is overcrowded with immature red cells, almost ready to released into the blood stream, but for some reason they are liberated. The decreased production of new cells may be accoun for by supposing a lack of a nutrient principle which is essential the maturation of the new cells, thus causing cessation of th development.

The results of Whipple and Robscheit-Robbins in their studies hemoglobin formation had shown liver and kidney to be especia valuable for this purpose. Acting on this suggestion, Minot a Murphy ('26) tested the therapeutic value of liver and kidney pernicious anemia. Their observations showed that in every one of cases the patients improved almost at once under liver therapy, a continued in good health as long as liver was regularly eaten sufficient quantities. The best results were obtained when raw li was eaten in large amounts, but patients experienced great difficu in consuming enough of it continuously to sustain normal blo regeneration. Subsequently Cohn and Minot et al. ('28) succeeded preparing a concentrate from liver which contained most of the acti principle and was much easier to ingest. Cohn and associates ('2 succeeded in fractionating liver to the extent that 140 mg. administer parenterally were clinically effective.

Having once developed pernicious anemia, it is necessary for t subject to continue taking the antipernicious anemia factor or compl during the rest of his life. It was difficult, therefore, to understand w normal persons who never ate liver or kidney did not devel pernicious anemia in a short time. Castle (Castle and Locke, '28) w

ressed with the fact that administration of liver or liver concentrate viated all of the major symptoms of the disease except one, namely, inability of the stomach to secrete hydrochloric acid and enzymes ch normally produce hydrolytic cleavage of the proteins of the d in gastric digestion. He postulated that the gastric secretion of normal individual contains an "intrinsic factor," which acting on "extrinsic factor," in association with the proteins of some foods especially abundant in meats, could develop a principle, "anti-nicious anemia factor," necessary for the maturation of the red od cells and bring them to a condition suitable for discharge into blood stream. His hypothesis implied, but did not specifically state, liver and kidney are simply storehouses of this latter factor which its origin in normal gastric digestion.

Castle tested this hypothesis by treating pernicious anemia patients n a diet of raw, ground muscle meat which had been predigested he stomach of a normal individual. Under this treatment all the rovements characteristic of liver therapy were observed. Feeding same kind of meat, without the previous digestion, produced no ission on other patients, but these responded well to liver therapy r. A third group of patients were given pure gastric juice obtained n normal persons. They did not benefit by this treatment. The clusion drawn from these observations was that the antianemia stance is not present in either meat or in normal gastric juice, but ne product of the interaction of these substances during digestion. The extrinsic factor has been shown to be present in a number of mon foods, among which are beef muscle, eggs, wheat germ, lyzed yeast, rice polishings and tomatoes. The nature of this ciple is still unknown. It was later demonstrated that when the ric mucosa of pigs was incubated with beef muscle the mixture effective in producing a hematopoietic response in patients with disease. Fresh gastric mucosa alone or whole desiccated stomach e, however, ineffective.

Greenspon ('36), on the basis of these observations, postulated the ry that "an erythrocyte-stimulating hormone resides in the gastric osa. In pernicious anemia, in which atrophy of the gastric mucosa s place, a loss of this hormone occurs, coinciding with the dis-arance of acid from the gastric juice. Thus, in addition to the -known external secretion (digestive), the glands of the gastric osa produce an internal secretion (hematopoietic); this suggests nalogy to the pancreatic gland. This hematopoietic hormone may rol the level of erythrocyte production in normal individuals."

Guided by this hypothesis Greenspon made experiments on one nt which resulted in the conclusion that pepsin is antagonistic to antipernicious anemia factor in stomach juice (Castle's intrinsic

factor). This view rested on the finding that pepsinized ventr was ineffective in pernicious anemia, whereas, feeding depepsi desiccated gastric mucosa was effective. The depepsinization accomplished by extraction of the pepsin from frozen gastric m using 2 per cent hydrochloric acid at 0° C. and precipitatin pepsin from the filtrate by means of acetone. The filtrate was combined with the residue so that a preparation containing a constituents of gastric mucosa except the pepsin resulted. Depepsi gastric mucosa, incubated with dilute hydrochloric acid, without tion of beef muscle, was effective in pernicious anemia. Feedi depepsinized gastric mucosa, incubated with dilute hydrochloric and pepsin, was ineffective. Feeding normal gastric juice, pept inactivated, was effective in causing remission of pernicious an without the addition of beef or other source of extrinsic factor. T observations are opposed to the existence of an extrinsic facto postulated by Castle.

Greenspon interpreted the normal process in the stomach follows: The beef (or other source of extrinsic factor) when i bated with normal gastric juice binds the pepsin and prevents it f inactivating the antianemia substance. He believes these find support the view, therefore, that it is no longer necessary to postu the existence of an extrinsic factor.

These findings have been controverted by Ungley ('36), Ca and Ham ('36), Helmer and Fouts ('37), and others, on the b of convincing observations that depepsinized gastric juice is ine tive in the treatment of pernicious anemia. The recent literature the value of different preparations has not clarified the problem the factors involved in remission of the disease.

The most notable attempts at purification and identification the antipernicious anemia factor (hypothetical reaction product the extrinsic and intrinsic factors) have been those of Dakin West. Dakin, Ungley and West ('36) believe that the substance or is associated with, a peptide, possessing many but by no me all the properties of an albumose." The evidence suggests that cosamine is not an active component of the molecule. The pept on hydrolysis, yields arginine, leucine, glycine, proline, hydroxyprol aspartic acid, and an acid resembling hydroxyglutamic acid. molecular weight probably is between 2000 and 5000. A few years the factor was believed to be a component of the vitamin B-comp and that it might be the substance now called riboflavin. Accord to Ashford, Klein and Wilkinson ('36) riboflavin is neither the a pernicious anemia factor nor the extrinsic factor. Trager, Miller Rhoads ('38) have reported that urine from normal persons patients with aplastic anemia or leukemia contains a substance wh

nhances the growth of mosquito larvae. But this substance is either cking, or present in much smaller amounts, in extracts from the ine of pernicious anemia patients. It is found in the urine of such ients after the institution of adequate pernicious anemia treatment. nce investigations of pernicious anemia are greatly handicapped the lack of suitable experimental animals, it would seem that mos-ito larvae might become of some use for this purpose.

Of interest are the studies of Williams, Macy et al. ('37) on the emical composition of the blood in pernicious anemia. They find e serum minerals to be unaffected, but the plasma lipids are char-terized by an increased amount of neutral fat and a deficiency of olesterol esters and phospholipids, which return to normal level ter therapy. According to their studies, the macrocytes of pernicious emia are slightly more spherical and the degree of sphericity, the ll volume, and corpuscular hemoglobin are related. Contrary to e prevailing belief, the chemical composition of the erythrocytes pears to exhibit striking abnormalities which indicate a deficient rpuscular structure. In relapse the red cells contain excessive amounts cholesterol esters and low amounts of phospholipid and free choles-rol. The cation and anion content of the red cells are elevated, e former due chiefly to increased potassium, and the latter to greater moglobin content. It is pointed out that physiologic activity of any sue or organ is associated with an increased content of phos-olipid and of free cholesterol, whereas lowered activity, degenera-n and retrogression are accompanied by a decreased content of se lipids and an increase in the amount of neutral fat and choles-ol esters. According to this view, the red cells in pernicious anemia in a state of lowered activity, or they are degenerating and rogressing.

Limited space does not permit further discussion of this subject. e literature on the nutritional aspects of pernicious anemia has been equately reviewed up to 1935 by West ('35).

Sprue.—Whereas pernicious anemia appears to be characterized nerally by a deficiency of the intrinsic factor, sprue is probably due, most instances, to a deficiency of the extrinsic factor in the diet complicated, perhaps, by an inadequacy of iron and perhaps some mponents of the vitamin B-complex, in addition to poor absorption the intestinal tract. This view is supported by the extensive investi-tions of Castle, Rhoads, Lawson and Payne ('35), whose observa-s show that the blood picture in sprue bears considerable resem-nce to the macrocytic anemia of pernicious anemia, and that sprue ndeed a deficiency disease. Also, the studies of Wills and associates ') indicates that the counterpart of tropical macrocytic anemia, ue, can be produced in monkeys by means of a diet devoid of

appreciable sources of the vitamin B-complex and low in
Wills et al. believe that the anemia is due to a deficiency of
extrinsic factor in addition to some other substance. Thus th
lem of nutritional anemias not involving iron or copper is
complex.

REFERENCES

Hirsch (1885). "A Handbook of Geographical and Historical Path
2, 492, The New Sydenham Society, London.
Robscheit-Robbins (1929). Physiol. Rev., *9*, 666.
Bunge (1889). Ztschr. f. Physiol. Chem., *13*, 399.
Abderhalden (1900). Ztschr. f. Biol., *39*, 193, 483.
Andral (1843). "Essai d'hematologie pathologique." Cited by Krur
(1937). "Pathology." Clio Medica, XIX. P. Hoeber, Inc., New Yo
Addison (1849). London Med. Gazette, *43*, 517.
Hart, Steenbock, Elvehjem and Waddell (1925). J. Biol. Chem.,
id. (1928), *77*, 777, 797.
Titus, Cave and Hughes (1928). J. Biol. Chem., *80*, 565.
Krauss (1931). J. Biol. Chem., *90*, 267.
Myers and Beard (1929). J. Am. Med. Assoc., *93*, 1210.
Mitchell and Miller (1929). J. Biol. Chem., *85*, 355.
Keil and Nelson (1931). J. Biol. Chem., *93*, 49.
Underhill, Orten and Lewis (1931). J. Biol. Chem., *91*, 13.
Cunningham (1931). Biochem. J., *25*, 1267.
Sheldon and Ramage (1932). Quart. J. Med., *1*, 135.
Davies (1933). Milk Industry, *13*, 55.
Elvehjem (1935). Physiol. Rev., *15*, 471.
Orten, Underhill, Mugrage and Lewis (1932). J. Biol. Chem., *96*,
Waltner and Waltner (1929). Klin. Wchnschr., *8*, 313.
Orten (1936). Am. J. Physiol., *114*, 414.
Barron and Barron (1936). Proc. Soc. Exper. Biol. & Med., *35*, 407.
Rominger, Meyer and Bomskov (1933). Ztschr. f. d. ges. exper. Me
786, 804, 809.
Kohler, Elvehjem and Hart (1935). Am. J. Physiol., *113*, 279.
Beard and Boggess (1935). Am. J. Physiol., *113*, 642.
Orten and Smith (1936). Yale J. Biol. & Med., *8*, 637.
Hogan and Ritchie (1934). J. Biol. Chem., *107*, 179.
Sebrell (1930). U. S. Pub. Health Reports, *45*, 1175.
Gruhzit (1931). Am. J. Med. Sci., *181*, 815.
Miller and Rhoads (1932-33). Proc. Soc. Exper. Biol. & Med., *30*, 54
Stucky and Brand (1932-33). Proc. Soc. Exper. Biol. & Med., *30*, 14
Day, P. L., Langston and Shukers (1935). J. Nutr., *9*, 637.
Suzman, Muller and Ungley (1932). Am. J. Physiol., *101*, 529.
Witts (1936). Lancet, *i*, 1.
Schmidt (1928). "Der Einfluss eisenarmer und eisenreicher Nahrun
Blut und Körper." Fischer, Jena.
Lintzel (1931). Ergebn. d. Physiol., *31*, 844.

VEHJEM (1932). J. Am. Med. Assoc., *98*, 1047.
ILL (1930). Proc. Roy. Soc. London, Series B, *107*, 205.
ERMAN, ELVEHJEM and HART (1934a). J. Biol. Chem., *107*, 383.
SE, VAHLTEICH and MACLEOD (1934). J. Biol. Chem., *104*, 217.
LE and BING (1936). Proc. Soc. Exper. Biol. & Med., *35*, 453.
RGEN and ELVEHJEM (1937). J. Biol. Chem., *119*, 725.
NTZEL (1933). Biochem. Ztschr., *263*, 173.
ATEN, SMITH and MENDEL (1936). J. Nutr., *12*, 373.
AY, H. G., and STEIN (1938). J. Nutr., *16*, 525.
OCK and DIAMOND (1934). J. Pediat., *4*, 442.
EOBALD and ELVEHJEM (1935). Am. J. Physiol., *111*, 118.
AVIDSON, FULLERTON, HOWIE, CROLL, ORR and GODDEN (1933). Brit. Med. J., *i*, 685.
SEPHS (1936). Medicine, *15*, 307.
ETTIER, MINOT and TOWNSEND (1930). J. Am. Med. Assoc., *95*, 1089.
ETTIER and CHEW (1931). Proc. Soc. Exper. Biol. & Med., *29*, 11.
NLOP and SCARBOROUGH (1935). Edinburgh Med. J., *42*, 476.
EARNS and MCKINLEY (1937). J. Nutr., *13*, 143.
AHN and WHIPPLE (1936). Am. J. Med. Sci., *191*, 24.
SEPHS (1932). J. Biol. Chem., *96*, 559.
HIPPLE and ROBSCHEIT-ROBBINS (1936). Am. J. Med. Sci., *191*, 11.
ACKLETON and MCCANCE (1936). Biochem. J., *30*, 582.
ERMAN, ELVEHJEM and HART (1934b). J. Biol. Chem., *107*, 289.
VEHJEM, HART and SHERMAN (1933). J. Biol. Chem., *103*, 61.
DANSKY (1921). J. Biol. Chem., *48*, 361.
HARGUE (1925). Am. J. Physiol., *72*, 583; id. (1926), *77*, 245; id. (1928), *8*, 637.
NDOW, ELVEHJEM and PETERSON (1929). J. Biol. Chem., *82*, 465.
VEHJEM, STEENBOCK and HART (1929). J. Biol. Chem., *83*, 27.
NDOW, PETERSON and STEENBOCK (1929). J. Biol. Chem., *84*, 419.
ULTZE, ELVEHJEM and HART (1936). J. Biol. Chem., *116*, 93.
LDON and RAMAGE (1931). Biochem. J., *25*, 1608.
OU and ADOLPH (1935). Biochem. J., *29*, 476.
HS, LEVENE and GRIFFITH (1937). Proc. Soc. Exper. Biol. & Med., *37*, 86.
EHJEM and SHERMAN (1932). J. Biol. Chem., *98*, 309.
IEN and ELVEHJEM (1934). J. Biol. Chem., *107*, 97.
EHJEM (1931). J. Biol. Chem., *90*, 111.
WSON (1935). Biochem. J., *29*, 569.
L and NELSON (1934). J. Biol. Chem., *106*, 343.
TER (1935). Nature, *136*, 185.
NN and KEILIN (1938). Nature, *142*, 148.
ULTZE, ELVEHJEM and HART (1936). J. Biol. Chem., *115*, 453.
EHJEM and HART (1929). J. Biol. Chem., *82*, 473.
ARNS and STINGER (1937). J. Nutr., *13*, 127.
E, MCC. VAHLTEICH, ROBB and BLOOMFIELD (1930). J. Nutr., *3*, 229.
HSENRING and FLOR (1932). J. Nutr., *5*, 141.
EHJEM, SIEMERS and MENDENHALL (1935). Am. J. Dis. Child., *50*, 28.

Josephs (1931). Bull. Johns Hopkins Hosp., *49*, 246.
Elvehjem, Duckles and Mendenhall (1937). Am. J. Dis. Child.,
Daniels and Wright (1934). J. Nutr., *8*, 125.
Hodges and Peterson (1931). J. Am. Dietet. Assoc., *7*, 6.
Mackey (1931). "Nutritional Anaemia in Infancy with Special Re to Iron Deficiency." Med. Research Council, Special Report Seri 157, London.
Osgood and Baker (1935). Am. J. Dis. Child., *50*, 343.
Wintrobe (1930). Medicine, *9*, 195.
Davidson, Fullerton and Campbell (1935). Brit. Med. J., *ii*, 195.
Widdowson and McCance (1936). J. Hyg., *36*, 13.
Orr (1935). Proc. Roy. Soc. of Med., *28*, 469.
Farrar and Goldhamer (1935). J. Nutr., *10*, 241.
Leverton and Roberts (1937). J. Nutr., *13*, 65.
Gillett (1918). Am. J. Physiol., *47*, 25.
Ohlson and Daum (1935). J. Nutr., *9*, 75.
Leverton and Roberts (1936). J. Am. Med. Assoc., *106*, 1459.
Strauss (1934). J. Am. Med. Assoc., *102*, 281.
Beard and Myers (1933). Am. J. Physiol., *106*, 449.
Van Donk, Feldman and Steenbock (1934). Am. J. Physiol., *107*, 6
Schultz (1933). München. med. Wchnschr., *80*, 679; id. (1934), A Gynäkol., *157*, 110.
Smallwood (1936). Brit. Med. J., *ii*, 573.
Adair, Dieckmann and Grant (1936). Am. J. Obst. & Gynec., *32*, 56
Garry and Stiven (1936). Nutr. Abstr. & Rev., *5*, 855.
Corrigan and Strauss (1936). J. Am. Med. Assoc., *106*, 1088.
Coons, Schiefelbusch, Marshall and Coons (1935). Okla. Agric. E Sta. Bull. No. 223.
Toverud (1935). Acta paediat., *17*, Suppl. No. 1, 131, 136.
Macy and Hunscher (1934). Am. J. Obst. & Gynec., *27*, 878.
McGowan and Crichton (1923). Biochem. J., *17*, 204; id. (1924), *18*,
Davidson and Leitch (1934). Nutr. Abstr. & Rev., *3*, 901.
Hart, Elvehjem, Kemmerer and Halpin (1929-30). Poultry Sci., *9*,
Cook and Harmon (1933). Am. J. Physiol., *105*, 407.
Graham and Smith (1929). Report to the British Empire Marketing B London.
McFarlane, Fulmer and Jukes (1930). Biochem. J., *24*, 1611.
Minot and Murphy (1926). J. Am. Med. Assoc., *87*, 470.
Cohn, Minot, Alles and Salter (1928). J. Biol. Chem., *77*, 325.
Castle and Locke (1928). J. Clin. Investig., *6*, 2.
Greenspon (1936). J. Am. Med. Assoc., *106*, 266.
Ungley (1936). Lancet, *i*, 1232.
Castle and Ham (1936). J. Am. Med. Assoc., *107*, 1456.
Helmer and Fouts (1937). Am. J. Med. Sci., *194*, 399.
Dakin, Ungley and West (1936). J. Biol. Chem., *115*, 771.
Ashford, Klein and Wilkinson (1936). Biochem. J., *30*, 218.
Trager, Miller and Rhoads (1938). J. Exper. Med., *67*, 469.

IAMS, ERICKSON, BERNSTEIN, HUMMEL and MACY (1937). J. Biol. em., *118*, 599.
(1935). J. Am. Med. Assoc., *105*, 432.
LE, RHOADS, LAWSON and PAYNE (1935). Arch. Internal Med., *56*, 627.
S, CLUTTERBUCK and EVANS (1937). Biochem. J., *31*, 2136.

CHAPTER

X

Iodine and Its Relation to Thyroid Functi

ALMOST from the time of its discovery by Courtois, in 1811, has been recognized as an important element in the maintena health, particularly in relation to thyroid function. For various r to be discussed briefly in this chapter, cretinism, endemic exophthalmic goiter, and some other types of thyroid disturb have been the source of human distress from time immemorial. have been largely problems of diet, for laboratory experimen and human experience during the past century have shown tha cases of thyroid dysfunction are attributable to inadequate an of iodine in the diet. It is necessary, therefore, to consider he more important aspects of iodine in nutrition. Since the present tions of significance are grounded in the early human expe with thyroid diseases, some attention will be given to the his background before discussing the current opinions and data c subject.

Early Views on the Cause of Goiter and Cretinism.—H (1885), in his *Geographical and Historical Pathology,* gives a tended account of the history of endemic goiter and cretinism states that medical writings of antiquity contain many referen "tumors" of the neck. Undoubtedly the "tumors" were goiters. light of our accumulated information some of the early views bizarre. For example, Wharton, who gave the first anatomic scription of the thyroid in 1656, suggested that the gland fulfi cosmetic function in women since it gave grace to the conto the neck. Hinkmar's *Life of St. Remi* (5th century) states th disease was believed to be a punishment from God, and cites effected by touching the King's hand.

Endemic goiter was known from ancient times to be co

certain regions, usually mountainous districts far removed from sea. Writers of the first century A. D. mention endemics of goiter the Alps, and in the 13th century Marco Polo gave an account of prevalence in the Central Asia plateau. As exploration increased it ame evident that the disease was world-wide, but the general im- ssion of the type of regions in which it was likely to occur re- ned unchanged. Moreover, the prevailing view was that the cause in environmental factors and most probably in some abnormality he water.

The seriousness of goiter and cretinism as health problems nat- ly occasioned much speculation and attempts at inquiry as to r cause. These are all discussed at some length by Hirsch, whose unt of the diseases will be found well worth study. Among some the opinions were its relation to the geology and mineralogy of affected locality. It tended to occur more frequently and with ater severity in regions characterized by older geologic formations n in the newer. Also it tended to occur only in regions of sedimen- formations which are composed of detritis of older rocks. Several ters attributed the prevalence of goiter to the habitual use of water in calcium and magnesium, but no generalizations were found hold true. Neglect of personal hygiene, pressure on the thyroid nd, some unknown poison, an infective agent, heredity, and the ciency of iodine in air and water, were all brought forward as ses of goiter and cretinism. It is interesting to note that Hirsch, ough the year was 1885, states that "It would then remain a ques- , and a very doubtful one, whether iodine has a prophylactic er against goiter, as well as a curative."

Observations on the Relation of Iodine to Thyroid Function.— generally believed at present that almost from time immemorial, nt sponge was more or less a popular folk remedy for goiter and inism. However, Harington ('35) writes that "The earliest refer- to this treatment which I have been able to find occurs in the ings of Arnaldus Villanovanus (A. D. 1280)." Certainly medieval opean physicians were generally aware of the remedy for reference occurs repeatedly in their writings. But it was at least over 500 s after Villanovanus that any inkling was found of the specific tance which caused the beneficial effects possessed by burnt ge.

ollowing the accidental discovery of iodine in burnt seaweed, by rtois in 1811, Fyfe, in 1819, isolated the new element from the ge. About this time Coindet (1820), a physician in Geneva where r was very prevalent, although unaware of the isolation of ne from sponge, postulated that the element discovered by Cour- was the goiter-curative principle in burnt sponge. As stated by

Harington ('35, l.c.), "The success which attended the fir which Coindet made of his theory was dramatic; large goi long standing disappeared with incredible speed in response ministration of iodine, and the enthusiasm aroused by the was such that people took to carrying little bottles of the element hung round their necks like charms."

Boussingault, in 1825, stated definitely that "until now io the only specific known for goiter." But the immediate wave thusiasm, as is so often the case, was accompanied by injudicio of the new substance. In many instances entirely too large an of the element were used with the consequent production of hyperthyroidism, the baleful nature of which was soon noted. iodine therapy soon fell into some degree of disrepute, alt Coindet had pointed out that toxic effects could be avoided b crete regulation of the dosage.

During succeeding years evidence continued to accumulate indicated the relationship between iodine and thyroid functio 1833, according to McCay ('35), Boussingault described the val salt rich in iodine as a preventive of goiter. Chatin's extensive st about 1850, demonstrated a significant correspondence betwee amounts of iodine present in water and foodstuffs in different with the regional incidence of goiter.

Although by this time the work of Coindet, Boussingault, Pré Chatin, Koestl, Lombroso, and others, had clearly demonstrated definite goiter-curative effect of iodine, the adverse report of R in 1860, to the French Academy of Medicine, swerved the me thought of that time to unreasonable points of view and caused essential abandonment of iodine in the treatment of goiter. The of Rilliet's conclusions were the results from experiments in w iodine was given to large groups of school children in France. the amounts employed were immoderately high, Rilliet and o concluded that the toxic effects observed warranted the exclu of iodine from the armamentarium of medicine.

The subject remained controversial until Baumann (1896) covered that the thyroid gland, in comparison with other tissue very rich in iodine. Once again there was a revival of interest in io deficiency as the cause of goiter. The renewal of prophylactic therapeutic treatment with iodine was based this time on careful control of dosage. Consequently, iodine in goiter prever became firmly established and is now sound medical and nutriti practice.

In this country Marine and his associates began to study the ject soon after Baumann's important discovery. In studies of histological changes which accompany goiter, Marine and Will

found that a fall in the iodine store precedes any cellular ges. They demonstrated that pups born of a mother with three-hs of the thyroid removed, the mother being kept on a diet low dine, had enlarged thyroids with the histological changes char-istic of goiter. The same animal when fed iodine during a subse-t gestation, delivered pups whose thyroids were normal in all cts. Following this early work of Marine, McClendon, and others, number of investigators increased rapidly so that at present the ture is extensive and in general agreement that iodine is essential rmal thyroid function.

ffects of Iodine Deficiency.—The principal anatomical effect of e deficiency in experimental animals is thyroid hyperplasia, but highly purified with respect to this element have not been ed. It appears that the increase in size, incident to iodine de-cy, is essentially no different from that in normal growth of land. However, adenomata appear early in the goitrous thyroid. ould be remembered, of course, that the thyroid gland is perhaps most vascular organ in the body and that changes in size may ot only of considerable magnitude but rapid as well. As stated rr and Leitch ('29) ". . . the normal histological picture and ich vascular supply appear to indicate a mechanism by which ormal physiological requirements for the thyroid hormone are ied by colloid absorption over relatively small areas with the rvation of a relatively large store of colloid capable of rapid ization. This in turn indicates that the body from time to time make urgent calls on the thyroid for a supply of the hormone." hormone production is dependent upon an adequate amount ine, this element must be constantly supplied to maintain a nor-unctional state.

emphasized by Marine ('35a), ". . . hyperplasia indicates hy-ivity but not necessarily hyperfunction. Myxedema and cretinoid may occur in individuals and animals with typical hyperplasia. nore accurate to consider all functional hyperplasia as indicating e or absolute iodine deficiencies and colloid goiter as the re-d, resting and physiologically normal stage." The normal gland rs to be composed of many closed (ductless) alveoli, filled with dy-like material known as *colloid,* and lined with a single layer boidal epithelium. In 1901 Oswald noted that the iodine was ned in the colloid. Also, he found that the iodine content of yroid varied in general with the amount of visible colloid. These nships were amplified by Marine and Lenhart ('09) who d that the iodine store in general varied inversely with the of active hyperplasia and that in extreme degrees of thyroid plasia the iodine store was almost completely exhausted. The

relation of iodine store to histological structure of different is shown in the following table prepared by Marine ('35, l.c

RELATION OF IODINE STORE TO HISTOLOGICAL STRUCTURE
(Iodine in mg. per gm. of dried gland)

SPECIES	NORMAL	HYPERPLASTIC STAGE			COLLOI RESTING
		Early	Moderate	Marked	
Man	2.17	0.88	0.71	0.32	2.00
Dog	3.32	0.62	0.37	0.11	1.99
Sheep	2.47	...	0.40	0.01	3.00
Ox	3.46	1.65	...	0.19	...
Pig	2.51	1.10	...	...	2.35

As shown by Marine and Lenhart, enlargement of the th does not occur if the diet contains adequate amounts of iodine in iodine deficiency the gland begins to increase in size as soo its iodine content falls below 0.1 per cent of the dried gland tiss

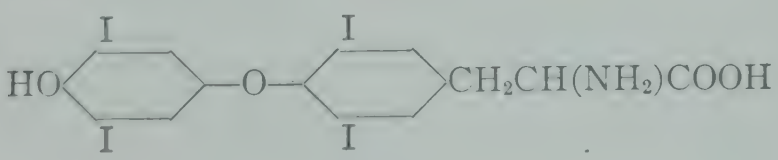
It is not profitable to discuss here the classifications of thy disease in medical literature; it suffices only to indicate the b types of the disease. These may be regarded as having their origi hypofunction or hyperfunction of the gland. *Myxedema* is a ge type of hypofunction. It may result from any factor which prev the adequate production of thyroid hormone, including, of co iodine deficiency. Reduced metabolic rate, thickening of subcutan connective tissue, alopecia, general physical and mental sluggish are the chief symptoms of myxedema, or deficiency of thyroid f tion. If the deficiency occurs in childhood, infancy, or in the bryonic stage, the condition is called *cretinism*. These conditions generally accompanied by an enlargement of the thyroid, or hy plasia. When the resultant swelling of the neck is noticeable called *goiter*. If the goiter is enlarged without hyperactivity it is ally referred to as *endemic* or *colloid goiter,* while if hyperacti does accompany it, the condition is called *exophthalmic goiter, Gra Disease,* or *Basedow's Disease.*

It is generally believed that exophthalmic goiter is attribut to hypersecretion of the thyroid hormone and that this may caused by excessive ingestion of iodine. Although excesses of io are very injurious, there is no conclusive evidence of this. It wo seem that the functional relationships with other glands of inte secretion are more important in exophthalmic goiter.

As pointed out by Evvard ('28), domestic animals, raised in areas iodine deficiency, may show marked degrees of iodine insufficiency. pigs, hairlessness is a common symptom. It is a peculiar mani- ation since the alopecia is rarely complete, many stages being resented in a single litter. The pigs are often of good size and generally carried *in utero* somewhat longer than normal. The skin thickened, pulpy, and very tender. Usually the thyroids are en- ged. At times colloid may be observed in the thyroids, but usually re is degeneracy, showing widely separated and irregular tubules h the intercellular spaces filled with serum, leucocytes, fibroblasts, avasated blood, and exfoliated follicular or acinic cells. These nptoms, and others, are quite variable but there seems to be no eral species-differences in the manifestations of iodine deficiency.

Compounds of Iodine in the Body.—The unique capacity of roid tissue to accumulate iodine is shown by Marine and Rogoff), who found that when 38 mg. of potassium iodide were admin- red to a dog by mouth, 18 per cent of the iodine was recovered from thyroid gland whose ratio to body weight was only 1:687. The rage normal iodine content of the human thyroid is reported to about 2 mg. for each gram of dried substance. The amount in er tissues is very much less, being expressed in γ per cent (1 γ = 1 mg.). It seems probable that all of the iodine in the thyroid is bined with globulin. The complex is commonly designated as *roglobulin*. According to Harington ('35, l.c.) the iodine com- , upon appropriate hydrolysis, can be accounted for between roxin and diiodotyrosine. He believes that ". . . the molecule of complete active secretion of the thyroid contains both thyroxine diiodotyrosine and that some linkage between these two is rup- d during hydrolysis which is a necessary initial stage in the isola- of thyroxine."

Thyroxin was first isolated by Kendall ('15). It was later syn- ized by Harington and Barger ('27), who gave it the following ctural formula:



(Thyroxin)

t has been known for several years that thyroxin is biologically active while diiodotyrosine is inactive. However, it is quite cer- that the hormonal function of the thyroid is not mediated, in rety at least, by thyroxin. As shown by Lunde et al. ('29), blood

iodine may be separated into two fractions by alcoh
Dodds, Lawson and Robertson ('32) claim that the insol
is increased in all patients with toxic goiter and that th
be reduced by orally ingested iodine.

Further investigation probably will reveal the more p
of iodine compounds in the body. Certainly one may c
the functions of iodine in bodily processes are not accompl
through the action of thyroxin alone.

Geographic Areas of Iodine Deficiency.—It is neces
sider the iodine content of soil and water since these ar
factors in determining the status of iodine nutrition in
mals. McClendon ('27) has written an extensive review
graphic distribution of goiter in relation to the amount
soil and water. Areas of notable iodine deficiency are
mountain regions of Southern Europe, including all S
Northern France, Northern Italy, and the Balkan States;
layan mountain region of Eastern and Southern Asia, the
trict of Northern India, and the plateau regions of Wes
and Eastern Mongolia; the Andes mountain region of Sout
the most noted section of which is the Peruvian plateau
New Zealand; and in North America, the entire basin of
Lakes, the basin of the St. Lawrence, various areas in the re
Rocky Mountains and the Pacific Northwest. In a well-kn
of British Columbia called Pemberton Meadows it was
raise farm animals owing to the marked deficiency of io
lowing the supplementation of foodstuffs with iodine, goi
to have practically disappeared from that region.

New Mexico, West Texas, Kansas, and the area north
South Dakota, are relatively rich in iodine. At one time
inland sea covered this region, which left vast salt depos
iodine. The Piedmont region of South Carolina also con
areas rich in iodine. But it is only in a few substances s
nitrate deposits of Chile and Bolivia, and in some sponges
other marine products, that concentrations as high as 0.1
cent occur. In McClendon's ('33) world survey, Japan wa
be the only non-goitrous country, there being about one
million Japanese. The relatively high consumption of seaw
is rich in iodine, is regarded as the cause for practical absen
from that country.

It is estimated that sea water contains about 25 parts
per billion. Seaweeds, sponges, and other marine plants ar
have great capacity for the absorption of iodine from sea wa
naria, a common seaweed, contains iodine equivalent to 0.

of its dry weight. Organisms in the soil, as well as the vegetation, absorb iodine and often contain much greater concentrations of this element than the rocks from which the soil was formed by weathering. The soil of glaciated regions contains little iodine, because the ice carried away the ancient soil with its vegetation and microorganisms, and the formation of new soil has not been in progress long enough to accumulate as much iodine as is needed by animals and man dependent on such soils for food. This is believed to account for the deficiency of iodine in much of Canada and the northern part of the United States.

There are certain other factors which deplete soils of iodine. Clay and humus soils tend to retain the element better than sandy soils or those poor in humus. Also, calcium is said to antagonize the retention of iodine since it disintegrates humus and renders the soil porous, in addition to its alkalizing effect. Remington found that potatoes grown on sandy soil contained little iodine, whereas those produced on clay soils were much richer in this element. Organic matter oxidizes rapidly in sandy soils, as pointed out by McClendon, thus liberating iodine. The problem of soil composition and physical status, particularly as respects the dispersion of components, is indeed important. Desirable factors for certain needs are undesirable for others.

Also, it is possible that the mode of combination of iodine in soils, as well as the amount, is important in determining the concentration of this element in foodstuffs. This is indicated by a report of the Medical Research Council of Great Britain (Committee on Iodine Deficiency and Thyroid Disease, '36) in which it was shown that the only appreciable difference between the iodine content of water, milk, and pasture grass of two different English counties was in the water. But this was not marked and scarcely explained the large difference in goiter incidence in the two counties. The observation suggests that the availability of soil iodine might be a significant factor in the occurrence of endemic goiter.

Use of Iodine in Prevention and Treatment of Goiter.—As pointed out previously, iodine prophylaxis was used as early as 1820, by Coindet, in conditions of iodine deficiency. But because of overdosage the early procedures were not very successful, and it was not until the present century that the administration of iodine, for such needs, became established on a wide and secure basis. The first large scale experiment in goiter prevention with iodine in man was begun in 1917 through the effort of Marine and Kimball ('17). They studied the effects of administering sodium iodide on the incidence and course of goiters in girls in the public high schools of Akron, Ohio. Two grams of the salt, in 0.2 gm. doses distributed over

a period of two weeks, were given to each girl. This proced
repeated each spring and autumn. Re-examination of the p
the end of the first year revealed practically the same drama
ventive and curative effects that had been noted in animals by
and other investigators. With the early results of this exp
available it became evident, as recently expressed by Marin
l.c.), that goiter is the simplest, easiest and cheapest of all kno
eases to prevent. The results of the study, at the end of the fir
are given in the table which follows:

	Pupils Taking Prophylactic Treatment		Pupils Not Prophyl Treatm	
	Pupils	Per Cent	Pupils	P
Thyroids remained normal	283	100	637	
Increased from normal to slight goiter	0	0	259	
Small goiters (unaltered)	287	66	759	
Small goiters (disappeared)	141	33.5	10	
Small goiters (increased)	2	0.5	103	
Large goiters (unaltered)	34	66.7	106	
Large goiters (decreased)	17	33.3	5	
Total	764	...	1879	
Total number of girls examined	4415	...	...	

In 1917 Kimball sent his first publication to Professor Klin
Zurich, Switzerland, who, in 1918, applied iodine therapy
school children of that city. In some schools 100 per cent of th
dren were goiterous. Klinger used an iodized fatty acid com
with chocolate and made into tablets. The method was late
ployed in the schools of the cantons of St. Gallen, Berne, and Z
with very striking results. Thus the value of iodine prophylax
been unequivocally established for at least twenty years, but the
of its usefulness has been extended by more recent investigation
most notable perhaps are those made in Michigan and Ohio. I
a special committee was appointed by the State Medical Soci
Michigan to cooperate with the Michigan State Department of H
in a state-wide program of goiter prevention. The State Depa
of Health made a goiter survey to determine the incidence of
in school children, and the amount of iodine in the water
of each section. The Wholesale Grocers' Association and th
Manufacturers' Association cooperated in putting upon the ma

able salt containing one part of potassium iodide in 5000 parts of alt, and in urging customers to buy this salt. The program con-emplated the recording of goiter incidence during a ten-year period, s shown by records of operations for goiter in hospitals, and by a e-survey of school children. The results of this grand experiment are ow available. They are best stated in the words of Dr. R. D. McClure '34), Chief Surgeon of the Henry Ford Hospital:

1. Iodized salt as used in Michigan did at first apparently increase the umber of thyroid operations.

2. The increase was in the nodular goiter or adenoma group, and we elieve the iodized salt may have activated a group of quiescent adenomata, roducing toxic goiter symptoms.

3. The increase reached its peak in the second year after the introduction f iodized salt.

4. An increase in the death rate from goiter as shown by the Board of lealth statistics reached its peak in the second year after the introduction f iodized salt.

5. There was no increase in hyperthyroidism, excepting in the nodular oiter or adenomata group.

6. The number of operations for toxic, diffuse and toxic nodular goiter as rapidly and steadily decreased after the apex of the second year increase ad been reached.

7. The incidence of endemic goiter or enlarged thyroid has been reduced most to nil since iodized salt has been so widely used.

8. We now see no cases which show the slightest ill effects from the e of iodized salt.

9. Toxic nodular goiter and toxic diffuse goiter are less apt to occur hen there has been no previous enlargement of the thyroid (endemic iter); at least this would seem a safe conclusion based on our experience.

As a basis for further evaluation of the Michigan experiment, e have the records from Ohio. In a significant paper by Kimball 7) the experiences of these two states in goiter prevention are ectively presented. It so happened that The Ohio State Department Health in 1925 made a rather comprehensive study of the in-ence of goiter throughout the state and planned to support the neral use of iodized salt. But owing to active opposition to this thod of prophylaxis on the part of some persons in the State, the blic health measure was essentially abandoned. Hence, it is en-ntening to compare the Michigan data with such of those which available from Ohio.

In 1936 a survey was made in the Cleveland parochial schools determine the incidence of goiter and the use of iodized salt. A dification of the tabulated results, given by Kimball, are as fol-'s:

	Total	Normal Thyroids	Goiter
Using iodized salt	2752	2503	249
Not using iodized salt	2785	1752	1033
Indefinite	798	624	174
Total	6335	4879	1456

In comparison with these data we have those from County, Michigan:

	Total	Normal Thyroids	Goiter
Using iodized salt	3089	3031	58
Not using iodized salt	1184	1009	175
Indefinite	618	593	25
Total	4891	4633	258

Thus, we have factual evidence that iodized salt, as a pro is effective. Moreover, there are other aspects of health than vention of goiter. Hypothyroidism or conditions of hyperp tivity, owing to iodine deficiency, are accompanied by defe than deformity of the neck. Mental development, sexual m and the development of positive personality are retarded iodine supplementation, insofar as it corrects or prevents a condition, promotes these functions, and contributes to the nance of good health.

Effects of Iodine in Large Amounts.—The advisability o iodine to the diet of man and animals has long been de shown earlier in this chapter. Unfortunately much of the co both pro and con, has had no basis in facts since clear-cut exp on the effects of iodine in various amounts have not been su numerous. At present, therefore, we are not yet certain as constitutes a "large" amount of iodine. Orr and Leitch conclude, on the basis of scanty evidence, that the minimu requirement may be about 45 γ for adults and 150 γ for chil the basis of this, one might presume that one hundred times, the stated requirements would constitute a large amount of

We know of no very useful objective studies on normal in this regard, but physicians have written extensively of thei

findings. We are not particularly concerned with such data and impressions here, since they are essentially medical problems and are concerned with the question of determining what types of thyroid disturbances should be treated with iodine.

The data from animal experimentation are illuminating. Evvard and Culbertson ('25) fed one group of pigs 40 mg. of potassium iodide per head daily for approximately 140 days. In this test and in two others of rather similar nature the iodized animals showed an increased growth rate and a reduction in the food consumption per unit of weight increase, as compared with control animals. In England, as stated by Orr and Leitch ('29, l.c.), pigs and brood sows fed as much as 1 gm. of potassium iodide per day showed no toxic effects. Phillips, Curtis and Erf ('34) have studied a herd of heavy-producing dairy cows which have been fed daily about 70 to 200 mg. of iodine, largely as potassium iodide, fish meal, and kelp, over a period of about 3 years. Physically the cows remained in very good condition. There was no evidence of hyperthyroidism, the milk and butter fat production increased, and the calves were normal.

There are instances, however, of severe injury when several grams of iodine are ingested daily, or even in one dose. The skin particularly is affected by eruptions and occasionally acute eczema develops. But one must conclude that the physiological range of iodine tolerance is fairly wide. Certainly no injurious effects can be attributed to the use of iodized salt, generous amounts of sea food, and other foods naturally rich in iodine.

Secondary Factors Contributing to the Cause of Goiter.—Marine ('35b) has pointed out that there are contributing causes other than deficiency in iodine, which may be relative or absolute. Puberty, pregnancy, the menopause, certain infections and intoxications, exposure to cold, excess of certain dietary constituents such as fats, proteins, and calcium, and deficient oxidation, all increase the needs of the body for the thyroid hormone which contains iodine. The effects of high calcium in the diet appear to be closely related to the levels of this element in proportion to the phosphorus and the magnesium content of the diet. Deficient oxidation may result from anemia or from oxygen deficiency due to living at high altitudes. Little is known about factors which may interfere with the absorption of iodine. McClendon has pointed out that organisms in the soil have a remarkable power to absorb iodides, as has been already mentioned. Hence, it is possible that when certain types of microorganisms, or certain animal parasites, constitute the prevailing flora or fauna of the alimentary tract the iodine taken in the food or water may be diverted to these and escape absorption. It has also been suggested that in certain cases the thyroid gland may have lost its power to utilize

iodine for the synthesis of the thyroid hormone, but this s is not given credence by Marine because it is not in harm his extensive observations.

That a high calcium intake may prevent the utilization by the thyroid gland is indicated by a number of observatio date from studies made over a century ago. Tanabe ('25), ('31), and Thompson ('33) have reported evidence which establish the claims that a high calcium intake increases th producing effects of certain diets. Zondek and Reiter ('23) fo the addition of calcium chloride to the water greatly del metamorphosis of tadpoles. Abelin ('28) found that when given calcium, the metabolic effects (stimulation of metabo of administered thyroxin was diminished. Rats restricted to what low iodine intake, and water containing a calcium salt, enlarged thyroids, while without excess of calcium the thy mained essentially normal. Thompson found that the inge calcium carbonate is increasingly effective as the iodine i lowered. The parathyroids are found to be enlarged in expe low-iodine, high-calcium feeding experiments.

Thus it is shown that there is more in goiter producti iodine deficiency. Several factors other than calcium have b vestigated but the results are essentially inconclusive. Some are fat, vitamins A, B-complex, C, and D. McCarrison ('3 others, believe that diets high in fat tend to produce goiter of the data regarding ascorbic acid (vitamin C) will be d later. It is not profitable to consider vitamins A, B-complex, until less equivocal results are reported.

Chesney, Clawson and Webster ('28) made the remarka servation that a diet consisting solely of cabbage may prod largement of the thyroid gland in rabbits. Clinically, de thyroid hyperplasia occurs in 2 to 3 months, and with co feeding of cabbage very large goiters (up to 45 gm.) were pr These goiters were associated with a lowering of the metabo and the administration of iodine raised the metabolic rate a vented thyroid hyperplasia. The thyroid hyperplasia produ feeding cabbage is histologically identical with simple or e goiter. These observations were confirmed by Marine and as (Baumann, Cipra and Marine, '31), and constitute definit that deficiency of iodine utilization and consequent thyroid plasia may be caused by some positive agent.

Marine and associates state that boiling or steaming cabbag minutes increases its capacity to produce goiter. The maximu of steaming is reached under thirty minutes. The fibrous mate

...ter pressing steamed cabbage is as effective as whole cabbage fed in equivalent amounts. The juice pressed from steamed cabbage is only slightly effective in causing goiter.

Rabbits previously iodized and fed fresh cabbage up to 75 calories per kilogram per day developed palpable thyroids in about 30 days, while with steamed cabbage the glands were comparably enlarged in 10 to 15 days. Rabbits normally have a metabolic rate of about 2.4 calories per kg. per hour, and after thyroidectomy, it falls to 1.75. This degree of lowering of metabolic rate was accomplished by feeding steamed cabbage in about half the time required when fresh cabbage was fed. Winter cabbage was more effective than summer cabbage. Steamed winter cabbage was effective in producing thyroid enlargement when fed in the amount of 25 calories per kilogram per day. Fresh or steamed carrots and mangel roots have no thyroid hyperplasia-producing qualities. Fresh turnip is ineffective, but steamed turnip is moderately goiter-producing. Brussels sprouts resemble cabbage in this respect.

Marine reasoned that since steamed cabbage is more goiterogenic than fresh, this property increases as the ascorbic acid content decreases during heating. Sterile fractions from the suprarenal glands, containing ascorbic acid, not only failed to produce goiter in rabbits, but had the opposite effect of causing involution. Marine concluded from the observations recorded that cabbage contains a powerful goiterogenic substance which acts by exhausting the thyroxin store of the thyroid gland. This is destroyed by enzymes in cabbage, but is stable when these are destroyed by heat. When present in the diet in sufficient amount, the goiterogenic substance rapidly depletes the iodine store, which would have been sufficient, in its absence, and hyperplasia results.

It was shown that cabbage is inactivated by drying in air at 25-35° C., or *in vacuo* at higher temperatures. Cabbage extracted with alcohol or acetone is quite inactive goiterogenically. The extracts, evaporated *in vacuo*, were likewise inactive. Prolonged boiling with access to air, or in acid or alkaline solution, or autoclaving, does not destroy the goiterogenic substance. Cabbage was extracted with ether and the fatty material obtained thereby was definitely goiterogenic.

Marine and associates tested the feeding of mustard oils (e. g., allyl isothiocyanate) to rabbits but with negative results. Injection of small doses of acetonitrile daily for 21 days caused striking thyroid hyperplasia, as did likewise hydrogen cyanide. Rats and mice were much less affected by the latter reagent than were rabbits but in spite of these numerous investigations the goiterogenic substance in cabbage has not yet been identified.

The Antigoiterogenic Effect of Ascorbic Acid.—The f heat treatment which destroys the antiscorbutic principle (acid) in cabbage likewise increases its goiterogenic proper gested to Marine and associates that ascorbic acid might hav tective, or antigoiterogenic action. They found that the ant genic property of fresh plant and fruit juices was in general i proportional to their iodine reducing capacity. They (Marine and Cipra, '32) maintained immature rabbits on a diet of alf and oats, which diet is goiterogenic, and showed that the goite effect was greatly enhanced by methyl cyanide. They show rabbits fed this diet, which were injected with the thyrotropic of the hypophysis, first prepared by Loeb and Bassett, and sim ously given 100 gm. per day of ascorbic acid by mouth, had t about 30 per cent smaller than those given the thyrotropic alone. The guinea pig thyroid reacts quickly to the thyrotropic and more slowly to ascorbic acid. The former, when both were being simultaneously administered, caused marked hyper of the thyroid by the fourth day. By the eighth day this ha appeared entirely, if the proper amount of ascorbic acid ha given. They found that ascorbic acid directly or indirectly coun the thyrotropic factor. It is apparent, therefore, that keeping the saturated with ascorbic acid is a useful procedure for prev iodine depletion, which may be brought about by several already mentioned.

Iodine Requirements.—As we have stated previously, the requirement appears to depend in considerable measure upo physiological status. Children, adolescents—particularly during p —and pregnant and lactating mothers, require considerably amounts than normal adults, if the higher incidence of goi such groups may be regarded as an indicator of relative needs mations based on "iodine balance" studies may be looked upon some skepticism. From the standpoint of cost it is essentiall possible to secure such data on more than a very limited numb persons. Moreover, the restrictions necessarily placed on subjects tested are such that the results can be scarcely regarded as repre tive of normal conditions. We are therefore without any very p information on the subject of iodine requirements. It is rather c however, that the range of iodine tolerance is wide. Hence, practice would be the free use of iodized salt (under the super of public health officials), sea foods, and other natural foods tively rich in iodine. Seaweed, according to McClendon ('33, has about 1000 times as much iodine as any other food. Since been a constant constituent of the Japanese diet for many gener and apparently without any injurious effects, it would seem tha

food might be used with impunity by people to whom it is table.

There has been some question of the utilization of different forms odine. Since this may be of some significance with respect to ne needs, investigations of the question are of definite value. nington, Coulson and Levine ('36), in studies on the rat, have wn that iodine in the form present in milk, oysters, and dried dock, was just as effective in the prevention of thyroid hypertrophy in the promotion of iodine storage in the gland as was potassium de. In general, the two forms of iodine were approximately of same value. Certain forms of iodine, however, are not very avail- in nutrition. For example, the iodine of iodosalicylic acid was rly utilized by laying hens (Asmundson, Almquist and Klose, . In view of the present evidence there is no real basis for con- ion that either "food iodine" or "inorganic iodine" is superior.

REFERENCES

SCH (1885). "Handbook of Geographical and Historical Pathology." ol. II, The New Sydenham Society, London.

RINGTON (1935). Lancet *i*, 1199.

CAY (1935). Science, *82*, 350.

MANN (1896). Ztschr. f. physiol. Chem., *21*, 319, 481.

INE and WILLIAMS (1908). Arch. Internal Med., *1*, 349.

and LEITCH (1929). "Iodine in Nutrition: A Review of Existing nformation." Special Report Series, No. 123, Medical Research Council, ondon.

INE (1935a). J. Am. Med. Assoc., *104*, 2334.

INE and LENHART (1909). Arch. Internal Med., *3*, 66; ibid., *4*, 253.

ARD (1928). Endocrinology, *12*, 539.

INE and ROGOFF (1917). J. Pharmacol., *9*, 1.

DALL (1915). J. Am. Med. Assoc., *64*, 2042.

INGTON and BARGER (1927). Biochem. J., *21*, 169.

DE, CLOSS and PEDERSEN (1929). Biochem. Ztschr., *206*, 261.

DS, LAWSON and ROBERTSON (1932). Lancet, *ii*, 608.

LENDON (1927). Physiol. Rev., *7*, 189.

LENDON (1933). J. Biol. Chem., *102*, 91.

mittee on Iodine Deficiency and Thyroid Disease (1936). "The Rela- nship of the Iodine Contents of Water, Milk and Pasture to the ccurrence of Endemic Goiter in Two Districts of England." Special port Series, No. 217, Medical Research Council, London.

INE and KIMBALL (1917). J. Lab. & Clin. Med., *3*, 41.

LURE (1934). Ann. Surg., *100*, 924.

ALL (1937). J. Am. Med. Assoc., *108*, 860.

ED and CULBERTSON (1925). Iowa Exper. Sta. Research Bull., No. 86, 3.

Phillips, Curtis and Erf (1934). Proc. Soc. Exper. Biol. & Med., *31*
Marine (1935b). J. Am. Med. Assoc., *104*, 2250.
Tanabe (1925). Beitr. z. path. Anat. u. z. allg. Path., *73*, 415.
Hellwig (1931). Arch. Path., *11*, 709.
Thompson (1933). Arch. Path., *16*, 211; ibid., *17*, 537.
Zondek and Reiter (1923). Klin. Wchnschr., *2*, 1344.
Abelin (1928). Biochem. Ztschr., *199*, 72.
McCarrison (1933). Brit. Med. J., *ii*, 671.
Chesney, Clawson and Webster (1928). Bull. Johns Hopkins Hos
261.
Baumann, Cipra and Marine (1931). Proc. Soc. Exper. Biol. & Me
1017.
Marine, Spence and Cipra (1932). Proc. Soc. Exper. Biol. & Med., 2
Remington, Coulson and Levine (1936). J. Nutr., *12*, 27.
Asmundson, Almquist and Klose (1936). J. Nutr., *12*, 1.

CHAPTER

XI

"Trace" Inorganic Elements

EMICAL ANALYSES of the animal body and of its products al the presence of a large number of the mineral elements, while trographic examinations detect traces of many others. Until recent s little significance has been attached to these "traces" of in- nic constituents. Whether certain of these are present because fulfill some physiological function or are incidental to the inges- of foods containing them is not yet known. Up to the present there is very little to indicate the part played by these physio- ally rare elements in human nutrition; therefore it is necessary iew the subject from the standpoint of animal nutrition. There wo aspects of the problem of trace elements in relation to health, e is that of the nutritional need of the animal organism, and other that which is manifested in industrial hygiene, agriculture, in foods, namely, the possibility of intoxication due to any one of minerals.

The mineral content of foodstuffs is subject to many determining rs,—the soil composition, effective rainfall, and other weather ents; for example, aridity appears to depress the phosphorus con- of crops and to raise the calcium content.

The continued feeding of rations deficient in one or more indis- able elements, either because of an injudicious selection of feeds, f abnormalities in composition of foodstuffs related to soil de- ncy, drought, or other cause, will eventually lead to the produc- of pathological symptoms. The excessive feeding of minerals may t in mineral malnutrition often of a severe type. The presence in s and water of toxic minerals, such as fluorine and selenium, also to mineral malnutrition when such materials are consumed in unts above certain safe minima.

Of the trace elements which are known to play physiologic and are indispensable in the diet, iodine, iron, and copper have been discussed. In this chapter will be considered manganes and cobalt, which appear to occupy positions of indispensabi addition, aluminum, strontium, vanadium, bromine, boron, and nickel, which are believed by certain investigators to play logical roles, but which appear not to have been definitely to be essential for normal nutrition, will be discussed, as fluorine and selenium. The nutritional importance of the la elements is open to question, but they are known to constitute hazards.

The efforts to secure undisputed evidence for the nutrition of any particular element are attended with much experimenta culty. This is particularly true when the element in questio be required by the animal in only small amounts. The experi approach involves the removal from the diet in so far as is p of the element to be studied.

Manganese

Manganese is universally distributed throughout the veg kingdom. The indispensability of this element for plant develop has been well known to experimental botanists for some years need for it is now recognized to such an extent that mangan included as a matter of course in a complete nutrient solutio water-culture work, although toxic effects may ensue if the am present is very large.

The occurrence of manganese in animal tissues and the exa of its necessity in the vegetable world indicated that mang might be needed by the animal body.

Several experiments had been recorded in which it was s to test the effects of adding manganese to diets not devoid o element, but without significant results. Richards ('30) and Pet and Skinner ('31) had indicated its significance for reproducti both plants and animals. Orent and McCollum ('31) made the crucial test of the need of this element by animals. They pre a diet extremely low in manganese, and observed that young ra this ration could grow to maturity in an apparently normal ma The females were shown, by the vaginal smear method, t through normal estrous cycles. They produced approximately normal number of young when mated with normal males. T females, deprived of manganese, failed, however, in 97 per ce the cases to suckle their young. They appeared to be indiffere them and did not give them the opportunity to suck which is

cteristic of female rats on the same dietary formula with small mounts of added manganese.

The manganese-free rats, when given foster young from stock tters, failed in 8 cases out of 10 to suckle them although the young ere vigorous and aggressive because of hunger. The 7 young reared y the other 2 females were retarded, apparently from insufficient milk upply.

Lactating female rats of the breeding stock did not show interest n and therefore did not give opportunity for the young of manganese-free mothers to suck. Only 7 of 107 manganese-free young were eared by stock foster-mothers. These were undersized and inferior in ppearance although the litters were small and in all likelihood secured all the milk they wanted.

Male rats raised on a manganese-free diet showed no abnormality ther than testicular degeneration. This degeneration is well under ay by the 100th day on the diet. The atrophy then rapidly progresses ntil only vestiges remain and complete sterility results. There was ttle or no obesity in these males. The same manganese-free diet, upon ne addition of 0.005 and 0.05 per cent, respectively, of manganese in ne form of the chloride, kept males in sexual potency for at least 14 onths. Their testes appeared normal at the end of the experiment.

The element was found to be absent, by the spectrographic test, om the young of manganese-free mothers. It is present in newborn nd 15-day old rats from mothers on manganese-containing diets; nce the element passes through the placenta if present in the other's blood. Manganese was spectrographically absent from the gans of rats raised on the experimental diet. It was suggested, from e results of these experiments, that manganese is in some manner ncerned with the production of a hormone by the anterior lobe of e hypophysis, which is essential for the functioning of the testes and r the normal development and functioning of mammary tissue.

Kemmerer, Elvehjem and Hart ('31) found that mice restricted to diet of whole milk supplemented with iron and copper, did not grow well as when 0.01 mg. of manganese per mouse per day was added. ithout manganese addition their mice did not ovulate normally, t when iron, copper, and manganese were added as supplements to lk the animals exhibited normal estrous cycles. In view of this parent species-difference, Orent and McCollum ('32) repeated their ork and confirmed their earlier observation that rats on a manganese-e diet exhibit normal estrous cycles and that deprivation of this ment had no appreciable effect on growth. Waddell, Steenbock and art ('31) attribute the somewhat slower growth rates and subnormal al weights of rats restricted to milk diets to a low caloric intake cause of the high water content of the diet.

Skinner, Van Donk and Steenbock ('32) and Van Donk, S bock and Hart ('33) reported that female rats receiving a milk diet supplemented with copper and iron did not exhibit n estrous cycles. Addition of manganese had no effect on the e cycle. Their animals on the milk-copper-iron-manganese diet failed to exhibit estrous over long periods of time and when did occur, they were less frequent than in the normal animal. Ho supplementation of the whole milk-copper-iron diet with sucro milk solids resulted in improved growth, ovulation, and reprodu in the rats. A deficiency of milk in energy for reproduction is the suggested by these experiments. Hence the difference in the ty diets may possibly explain such discrepancies as exist betwee observations of the two laboratories (Johns Hopkins and Wiscon In addition, it should not be overlooked that milk may be some deficient in unknown nutrients essential for development into normal adulthood which were provided in the diet of Orent McCollum. Also, Keil and associates ('34) showed that the nee manganese for growth or reproduction may depend to some e upon the store of manganese in the rats when the experiment is be These investigators obtained reproduction in first generations of fed milk supplemented with iron and copper, but the second ge tion failed to reproduce unless manganese was added.

Daniels and Everson ('35) employed a milk-iron-copper-manga diet on which they reared fifth generation young rats with app mately 100 per cent viability. The same diet (i. e., cow's milk) wit added manganese contained 0.03 mg. of this element per liter. On latter diet 8 first generation females produced 103 young; 47 in litters, 33 in second, and 23 in third. These young were sired by s males, since males on the manganese-deficient diet are sterile. Of t 103 young, 41 were either born dead or died within two hours were anesthetized for analysis; and 37 were transferred to s mothers. Sixty-nine per cent of the latter subsequently died with few days; 12 were raised by 2 females (6 each) to average wea weights of 53.7 and 41.5 gm. respectively. In marked contrast to t results were the young of fifth generation females restricted to m iron-copper-manganese. One hundred per cent of these were re with an average weaning weight at 28 days of 69.2 gm., a we corresponding to young of the breeding stock. The rats must h secured an adequate caloric intake from this particular liquid diet.

Mother rats on this manganese-deficient diet, which had lost t young at birth, were able to suckle 96.9 per cent of foster young f the stock colony to average weaning weights of 58.7 gm.

In 1935 Daniels and Everson (l. c.) using a milk-iron-copper containing 0.03 mg. manganese per liter, studied the cause of the h

ortality in the young born of female rats on this manganese-low et. These young were sired by stock males. More than a third of the oung of manganese-depleted mothers were born dead or died shortly ter birth. Those that did survive were not active enough to suckle en when transferred to stock mothers; for 69 per cent of them subquently died within a few days. However, mothers on the manese-low diet were able to suckle young taken from mothers on a rmal diet. The diet of Orent and McCollum was much lower in anganese than that of Daniels and Everson, which may account for e lower capacity of their rats to suckle stock young.

From Daniels and Everson's observation and those made by Orent d McCollum, it is evident that manganese plays an important role the development of the fetus during gestation. Fertility persists in male rats restricted to a diet so low in manganese that the young are t normally nourished *in utero,* and are debilitated at birth.

Tutt ('34) described 3 cases of sterility in cows, in 2 of which arian cysts were found. Injections of colloidal manganese were given d pregnancy followed in 2 animals. In the third, administration of anganese and wheat germ oil were unsuccessful.

It is of interest that attempts to induce symptoms of manganese ficiency in pigs have not been successful even though the concention of manganese in the ration was reduced to 1/17 of that of a rmal ration and the feeding was continued for 10 or 11 months. It pears that the manganese requirement of the young growing pig satisfied by a concentration of 1 part in 180,000 of the ration. If ury from manganese deficiency is to be produced in this species, it ll be necessary to reduce the manganese content of the ration still rther.

The role of manganese in the nutrition of birds has recently asmed practical importance. Wilgus and coworkers ('37) have shown t a bone abnormality, called perosis, first described by Hunter d Funk ('30) and by Payne ('30), can be almost entirely prevented addition of 0.0025-0.015 per cent of manganese to a diet on which disease regularly occurs in a high percentage of chicks although lready contains 0.001 per cent of this element. The symptoms of this order are gross enlargement of the tibial-metatarsal joint, twisting bending of the distal end of the tibia and proximal end of the tatarsus, and slipping of the gastrocnemius tendon from its conles. The latter incident causes severe crippling in the affected leg d if both legs are so affected, death soon follows from inability to food. Excess of calcium and phosphorus in the diet tends to insify the disease, whereas it has been shown by Titus ('32) that the icarp of rice, oats, and wheat, tends to prevent it. This action is w attributed to their high content of manganese. Wilgus and asso-

ciates (l.c.) found that zinc and aluminum possessed a similar ventive property, but were less effective than manganese. It w appear that these elements exert their effect through binding phate in the intestine and preventing the formation of inso and unabsorbable manganese phosphate. A mixture of manga aluminum, and iron was shown to be entirely preventive in presence of limited amounts of calcium and phosphorus. F the evidence now available it appears that deficiency of mangane the primary cause, but that excessive feeding of calcium and p phorus aggravates the condition, and such preventive action as c elements may afford is through their influence on the absorptio excretion of manganese, calcium and phosphorus.

Lyons and Insko ('37) observed that eggs from hens which been fed a manganese-deficient diet gave a hatchability of less tha per cent. Those embryos which were sufficiently developed for servation before death showed without exception very short legs, did not develop slipped tendon. Another group fed the same ra plus 40 p.p.m. each of manganese as sulfate, and 100 p.p.m. of as ferrous ammonium sulfate, produced eggs giving good hat bility and normal chicks. Embryos which died showed normal velopment of appendages and mandibles. Eggs from hens fed perosis-producing diet contained much less manganese than th from hens fed the supplements named. These observations sugges manganese deficiency as the cause of the abnormality. This was pro to be correct by the injection of 0.03 mg. of manganese directly i the albumen of such eggs just prior to incubation, which resulted an increase in hatchability and the normal development of the e bryos. The metatarsi, tibiae, and humeri of chicks and 20-21 day e bryos from the injected eggs were 52, 44, and 40 per cent long respectively, than the same bones from the eggs which were not jected with manganese. The injection of a similar amount of zinc s into the eggs had no preventive effect on the disorder. These resu have been confirmed and extended by the studies of Gallup a Norris ('38).

Skinner and associates ('31) found 0.038 and 0.034 mg. of m ganese per 100 gm. in the shaft and head, respectively, of fresh bone, with only traces in the marrow. By feeding a diet high in m ganese they could increase the content of this element to 0.221 m Gallup and Norris ('38, l.c.) found 0.060 mg. per 100 gm. of dry mat in chick bones from a manganese-deficient diet, whereas by supp menting the diet with this element they raised the content in bone a fairly constant value of about 0.200 mg. Partial depletion of the bo in manganese resulted in the characteristic deformities.

Lyons, Insko and Martin ('38) studied the manganese requi

nt of the growing chick. They found that the minimum protective l of manganese under the conditions of their experiment appears e 35—40 p.p.m. of manganese. These investigators state that the t minimum protective level of the element cannot be fully estab- d until the relationship between the calcium and phosphorus of diet and the availability of manganese is known. They suggest that e may be three main factors which determine the protective level anganese in the chick's diet: (1) the level of available manganese he diet of the mother hen; (2) the calcium and phosphorus con- of the diet; and (3) the availability of the manganese supple- t.

From these studies it appears that the amount of manganese which sential for normal development is greater for birds than for mam- , and also that skeletal defects are produced in birds which do not ar to occur in the rat. This statement is based only on the observa- of Orent and McCollum that their rats on a manganese-deficient grew normally, and were long and normally lithe animals. No t observations are available showing the histological changes, if in bones of such rats.

Nothing definite is known about the human requirement for man- se. There appears to be no evidence that manganese deficiency rs in man, but it is quite possible that it may do so. The experi- ts of Everson and Daniels ('34) on children, which followed their al studies, are of interest from that standpoint. Their results in- e that there is a definite manganese need in children, which may e met by diets that appear adequate in all other respects. The unt of manganese retained was found to be proportional to the unt ingested, thus indicating that manganese is essential to the iological development of children. They suggest, therefore, that iet of children should contain between 0.20 and 0.30 mg. of man- se per kilogram of body weight.

hronic manganese poisoning has been recognized both in man animals. The toxic effects observed in animals are only with doses rge as to cause gastric disturbances. These effects include degen- e changes in the central nervous system, liver, and other organs, disturbances of the circulatory system.

ecker and McCollum ('38) report that on purified diets supple- ed by 0.0499, 0.0998, 0.2495, and 0.4990 gm. manganese, rats grew and produced healthy young. On a diet supplemented with gm. of the mineral, there was some depression of growth. Re- uction, however, was excellent. No symptoms of manganese ty were observed. Evidently the high phosphorus content of the revented such symptoms by reducing the amount of absorbable anese.

Zinc

Zinc has been shown by a number of investigators to essential element for the development of plants. However, ov its wide distribution in foods, it is unlikely that zinc deficien occurs in animals except when induced experimentally. That occur naturally in plant life has been observed by Finch an nison ('33).

Zinc occurs in practically all animal tissues but is most ab in the liver and pancreas. Only traces of this element are fo lung, brain, or testicle. The zinc content of blood is markedly v Colostrum contains three times the amount of zinc present in milk, and the zinc content of active mammary gland is twice as that of the inactive gland.

The earliest attempt to find whether zinc is important in nutrition was made by Bertrand and Berzon ('22), who, ho employed a diet deficient not only in zinc but in several nutrient tials. They noted that rats receiving zinc survived 25 to 50 pe longer than their zinc-low controls. Hubbell and Mendel ('2 served that mice throve better with added zinc than did their co Newell and McCollum ('33) could not reduce the zinc content o low enough to show that this element was important, but Elvehjem and Hart ('34) were more successful in this respect, h prepared a diet containing but 1.6 mg. per kilogram of food. Ad of zinc to this diet, which was supplemented with 2 cc. of mil rat per day improved the growth of the animals. Stirn, Elvehjem Hart ('35) have reported that zinc is an essential element in nutr The only effects produced by deprivation of this element which described were retardation of growth and interference with th velopment of a normal coat of hair. More recently Hove, Elve and Hart ('37) pointed out that there are some indications of def intestinal absorption in rats on zinc-low diets.

An interesting suggestion concerning the possible action of came from the work of Scott ('34), who showed that zinc w constituent of insulin crystals. In view of Scott's clear-cut evidence cerning the rather unique effects of zinc salts on the formatio crystalline insulin, and the fact that the pancreas is very rich in this element may play a significant role in carbohydrate metabo However, a definite interpretation as to how zinc functions ca yet be made. At present there is no definite relation known bet zinc and insulin activity.

Maxwell ('34) found that the presence of small amounts of salts in hypophyseal extracts produced a marked augmentation i ovarian weight increase in young female rats. He also observed

mentation with pregnancy-urine preparations to be increased when salts were added to the hypophyseal synergistic component. Hove, ehjem and Hart ('37, l.c.) also have suggested a relationship be- en zinc and the hypophysis. At present there is but little basis for postulate. Until a better dietary procedure is developed for the duction of zinc deficiency it will be impossible to make positive rtions regarding the indispensability of this element.

Cobalt

The demonstration of the biological significance of cobalt is of the most interesting of recent developments in nutritional re- ch. McHargue ('27), Fox and Ramage ('30), and others reported ing traces of cobalt in biological materials, but Stare and Elvehjem) stated that if this element occurs in animal and plant products es so in extremely small amounts. They found less than 0.01 mg. balt in 100 gm. of milk. Blumberg and Rask ('33) made a spectro- hic analysis of the ash of milk and could not detect cobalt lines ne spectrograms. Stare and Elvehjem found less than 0.01 mg. of lt in the entire body of rats reared on milk supplemented with copper, and manganese. The amount in the organs of young e reared on the same ration was of a similar order.

Valtner and Waltner ('29), while studying the toxicity of various ls, found that a polycythemia was produced when either powdered llic cobalt, cobalt chloride, or nitrate in amounts of 0.5 to 2.0 per was added to the mixed diet of normal rats, or when 0.01 or 0.1 of cobalt chloride or cobalt nitrate was injected subcutaneously. fact was corroborated by Orten and associates ('32). The follow- able illustrates the effect on the blood of administration of cobalt:

D FINDINGS IN RATS FED MILK-IRON-COPPER DIET SUPPLEMENTED WITH COBALT SULFATE

OUP NO.	SUPPLEMENT TO MILK-Fe-Cu DIET	BODY WEIGHT	HEMOGLOBIN	CELL VOLUME	ERYTHROCYTES
		gm.	*gm. per 100 cc.*	*per cent*	*per c.mm.*
. . . . .	None	236	12.2	52	7,900,000
. . . . .	$CoSO_4$	180	17.0	73	10,700,000

rten and associates ('32, '33) observed in cobalt polycythemia ite rise in blood volume, which was due to increased cell volume r than to significant change in plasma volume.

Feeding manganese with the milk-iron-copper-cobalt diet se
alleviate the toxic effects resulting from prolonged administr
small amounts of cobalt. It also seemed to increase the effect
in raising the red cell count, which the authors express as "som
izing influence on the increased hemoglobin, erythrocyte, cell
and blood volume values characteristic of cobalt polycythemia

Little information is available at the present time regar
mechanism involved in the production of polycythemia by
Preliminary work indicates that the spleen does not play an
role in the phenomenon.

Ascorbic acid seems to assist in the maintenance of a
level of red blood cells in the circulating blood. When ascor
was injected intravenously, simultaneously with cobalt, into
polycythemia failed to appear; when ascorbic acid was injecte
the production of polycythemia, a decrease in the hemoglobin
tration and red blood cell count resulted, but the effect was tem
According to these investigators (Barron and Barron, '36),
polycythemia seems to be due to the inhibition by cobalt of
spiratory function of immature red blood cells.

***"Coast Disease," "Bush Sickness," "Pines," or "Enzootic
mus" in Farm Animals.***—In New Zealand, Australia, Florid
certain localities of the North Gulf and South Atlantic states, th
long occurred a disease of sheep and cattle known as "coast d
"bush sickness," and "wasting disease." The condition was de
in 1896 by Aston, in New Zealand, as a disease affecting cattl
animals become greatly emaciated, primarily as the result of
appetite, and suffer progressive anemia. The anemia suggeste
deficiency, and analysis of the soils and herbage in areas wh
disease occurred showed that they were very low in this elemen
provision of iron licks, in the form of limonite, a hydrated ferric
in some instances afforded complete relief to the animals, but i
cases it was less effective or not at all. After some years of study
shown by Underwood and Filmer ('35) that an iron-free prep
made by acid extraction of limonite, was effective, and the proph
value of the extract was traced to its content of cobalt. This w
first evidence that cobalt is an essential nutrient for mammals.
ton ('35) and Lines ('35) reported successful cures of sheep su
from "bush sickness" by means of pure cobalt salts. Lines ('3
Askew and associates ('36, '37), and Dixon ('36) have extended
studies, and have fully confirmed the view that deficiency of
in soils and in herbage grown on deficient soils is the cause
disease. The cobalt requirement of sheep (Lines, '35, l.c.) is
ently about 1 mg. per ewe daily. Denham ('37) has fully review
investigations in this field.

Neal and Ahmann ('37) have pointed out that in Florida, on certain types of soil, ferric citrate or iron oxide and copper sulfate were found effective in preventing "salt lick," an anemia occurring in cattle. On other soils, these supplements were not sufficient because of deficiency of cobalt as well as iron and copper. They found that calves on a ration of Natal grass hay, corn, and dried skim milk developed anemia, and were cured by cobalt administration.

They describe the symptoms in cattle to be long, rough hair, scaliness of the skin, listlessness, gauntness due to loss of appetite, muscular atrophy and retarded development of sexual characteristics. The erythrocyte count may be above average, and the hemoglobin concentration equal to or above that in animals receiving cobalt and growing normally. Volume and color indices show that the condition is a macrocytic hypochromic anemia. The spleen is shrivelled and fibrous, the heart flabby, although of normal size. They describe growth studies in calves fed Florida-grown feeds, and show that supplementing these rations with cobalt is essential to successful animal production in this region.

The studies described illustrate how elusive may be the cause of malnutrition in animals or in man in certain instances. The discovery of the indispensability of cobalt is an example closely paralleling the problems in animal and poultry production arising in certain regions from iodine and from manganese deficiency.

Nickel

There is at present no definite evidence concerning the significance of nickel in animal nutrition.

Nickel is present in some foodstuffs. Blumberg and Rask ('33, l.c.) did not detect it spectrographically in cow's milk. Newell and McCollum ('31) reported its occurrence in certain edible fish. Bertrand and Macheboeuf ('25) found nickel in brain, skin, spleen, pancreas, and liver. The fat, muscle, and uterus contained but small amounts of the element. Dutoit and Zbinden ('30) found it by spectrographic analysis in the ash of human blood and they also observed its tendency to accumulate in the human pancreas. Fisher and Scott ('35) were able to detect nickel in the pancreas of cattle of any age. They state that possibly only traces of these metals are present and that the amount is beyond the sensitivity of the method used in their studies.

According to Bertrand, insulin preparations contain a considerable amount of nickel. He showed that the simultaneous injection of a small quantity of nickel and insulin into a rabbit or a dog greatly increased the action of the insulin on the blood sugar. Blatherwick and Sun ('27) did not confirm these findings. They found that there

was a great deal of individual variation in the insulin hypog resulting from a certain dose per kilo of body weight. They that the results obtained by Bertrand might have been due variation rather than the metal.

Boron

Boron has been demonstrated to be essential for plant g but there is no experimental evidence in support of the vie it is necessary for animals. Data on the distribution of bo animal tissues is scant. Bertrand and Agulhon ('13) reported th ence of this element in various tissues of the guinea pig, rabbit, ox, and horse. Boron was also found in human, cow's (Wrig Papish, '29; Blumberg and Rask, '33, l.c.), and ass's milk, as v eggs of fowl (Drea, '35), turkey, and goose. This mineral el appears to exist normally in small amounts in all animals, being common in the species of marine origin.

McCollum and Yue ('37) studied the distribution of boron i of their stock colony fed the stock diet, in rats from this same c fed purified diets containing varying amounts of boron, and a pathological organs and tissues of humans. They observed tha boron content of the whole rat of their stock diet increases with and varies with the size of the animal. The results of these experi indicate that this element is transmitted to the young throug placenta. Newborn young contain a fairly high concentration of b but the element seems to decrease during the nursing period, w leads to the suggestion that the milk of these rats is probably in boron.

Boron is invariably found in all the tissues of the adult rat; and skin being the richest locales. The boron per cent in muscle the skeleton is always low, but it seems that some is always ent. The boron content of the hair not only increases with but also varies with sex and the concentration of the element i diet. The diet plays a major part in determining the boron co of the rat. Most of the boron ingested is excreted and but a amount is retained by the animal organism.

In pathological tissues of human subjects, boron is present in n all the organs and tissues. As in the rat, the accumulation of thi ment is greatest in the hair. Heart, stomach, lung, and kidney fo in the order named. Age seems to be an important factor in regul the concentration of boron in both animal and human tissues. amount of the element in the human body increases with age varies with individuals. Apparently the origin of boron in the an body lies in the food ingested.

Bromine

Bromine is an invariable constituent of marine algae. Land
nts contain considerably less of this element. Organs and tissues
ll marine and land animals contain it, but in variable amounts.
s thought that the actual bromine content of any tissue is de-
nined by environmental conditions and perhaps selective affinity
he species and cells in the different parts of the individual tissue.
The metabolic significance of bromine has been discussed by a
nber of investigators. Bertrand ('20) suggests that this element may
as a catalyzer in the body. Various statements (Ucko, '34; Ku-
ami, '32, '33; Bernhardt and Ucko, '26; Zondeck and Bier, '31, '33;
bson, '35) have been made that the anterior pituitary gland is con-
ed with the metabolism of bromine. These findings, however,
e been contradicted by Serbescu and Buttu ('34), Dixon ('35), and
feld ('36). The amount of bromine normally present in the
itary is of the same order as that of the blood. At this time there
nsufficient evidence for the belief that the pituitary plays an im-
ant role in the physiological function of bromine.
Tanino ('31a, '31b) and Jacobson ('35, l.c.) have considered a pos-
e relation between the thyroid gland and bromine metabolism.
y believe that the thyroid probably produces a bromine-containing
stance, possibly dibromthyroxin. Neufeld ('36, l.c., '37) has shown
the thyroid contains amounts of bromine somewhat higher than
e present in other tissues. The Br:Cl ratio in mammalian tissues in
feld's experiments does not show sufficient constancy to suggest the
tence of any physiological ratio. However, the ratio for the thyroid
latively much greater than for other tissues. A larger number of
yses are needed before attaching significance to this finding. From
experimental evidence available it is not yet possible to state that
mine plays a role in the physiology of this gland. Neufeld's results
cate that it is not functionally associated with the essential prin-
e of the thyroid. Further investigation is necessary before any
tional significance of bromine to the thyroid gland is ascertained.
Winnek and Smith ('37) studied the nutritional effect of synthetic
s containing 5 p.p.m. bromine. They observed no particular differ-
e between the general appearance, rate of growth or reproduction
he animals on the bromine-low diet, and the diet supplemented
the element. There was no evidence that bromine is essential in
nutrition of the rat. The amount of bromine in the tissues of these
nals depends not only on the amount of bromine ingested, but
on the Br:Cl ratio of the diet. The young born to mothers on the
mine-low diet have a greatly diminished bromine content as com-
d with young born to the stock females.

Silicon

Next to oxygen, silicon is the most abundant element in It is found in plants and in many marine organisms. I amounts it appears to be a natural constituent of all animal ti is present in hair, skin, thymus, tendon, dura mater, and len eye. The silica content of the individual organs appears to be dependent upon their connective tissue content (Schulz, '01 nerman ('18) determined the silica content of bile, blood stomach, intestine, liver, kidney, heart, brain, testicle, skin, ar He ('20) also found silica in various concretions, some of whic of human origin; they included biliary calculi, urinary calculi, b and enteroliths. This mineral has been found in milk (Wrig Papish, '29, l.c.; Drea, '34), in edible fish (Newell and McC '31, l.c.) and in all other tissues from normal chicks (Drea, '3 Brown ('27) obtained quite variable results for the silicon c of human skin. A definite, but not marked, tendency apparen isted for a decrease in the silicon content of the skin in older p King and coworkers ('33) have detected small but significant an in fetal tissue. They also showed that in adult animals a consid excretion of silicate occurs through the kidneys and that the c tration of soluble silicates in the urine is influenced by the diet. the urinary excretion of silica was markedly increased there w significant change in the blood silica, except slight temporary inc indicating an extremely low renal threshold for silicates. At p there is no experimental evidence to show whether silicon is a n constituent of protoplasm or whether its occurrence is merely a titious.

Strontium

Stoeltzner ('08) and Lehnerdt ('10) observed that feeding tium led to the formation of excessive amounts of osteoid tiss bones.

Wheeler ('19) found that strontium was capable of replacin cium to a considerable extent in the egg shell and in the bones. eggs were obtained from hens fed strontium salts than those fe cium salts.

Shipley and coworkers ('22) pointed out that this mineral c replace calcium in bone formation. Kinney and McCollum ('23) that the entrance of strontium into the bones was not inhibited b presence of calcium in the diet. Lesné, Vagliano and Christou confirmed these observations.

Drea ('35, l.c.) showed that strontium was one of the ele that pass from the feed and water into the hen's blood and egg

en into all of the chick's tissues and the chick's blood. Drea believes ese findings to be of definite physiological significance. He suggests at this distribution of strontium may be due to the inability of the n's organism to distinguish it from calcium.

Vanadium

There is little information about the effect of vanadium in the et. The few studies reported have dealt with the toxic action of nadium salts and most of these have been pharmacological experi-ents.

Vanadium is one of the "trace" elements in the hen's blood and g. It is present in significant amounts in the blood of the chick d hen, in the femur, heart, kidney, and lung. Drea ('35, l.c.) believes at this suggests some function associated with the hemapoietic sys-n.

Several French experimenters (cited by Lyonnet et al., 1899) have orded the therapeutic effects of small doses of vanadium in various eases.

Daniel and Lillie ('38) in a study of the nutritional significance of s element report certain basic data as to tolerance levels of vana-m in the diet and the histologic effects produced by the ingestion the element. They fed the mineral in the form of sodium tavanadate in the following amounts: 11.5, 23, 92, 184, and 368 .m. respectively. The animals on the diets containing 11.5 and p.p.m. vanadium showed no toxic symptoms. On the diet containing p.p.m., death of the rats occurred. In the amounts fed in these ex-iments they noted no significant indication of a stimulating effect of all amounts of vanadium. There was no evidence of a cumulative ct from vanadium ingestion.

With the high concentration of vanadium, the food consumption he rats decreased and starvation was superimposed on the condition chronic poisoning. A definite symptomatology and pathology was duced by the higher levels of vanadium in the diet.

Aluminum

Aluminum is universally distributed in soils and plants, and mals have been in intimate contact with this element through-the ages. Numerous analyses of plant and animal tissues have n recorded in the literature, but whether or not uncontaminated ues from either class of substances contain appreciable amounts aluminum is still a matter of dispute. It is generally agreed t the amounts present, if any, are very small. There are two types

of analytical procedure for its estimation in extremely small qua
(1) the spectrographic method based upon the number and int
of lines in the spark or arc spectrograms. As the amount of alu
in the sample decreases to extremely small amounts, certain lin
out, and the results may be made roughly quantitative by com
lines from the material under examination with those of sta
prepared with known amounts of the element. (2) The other r
depends upon the ability of aluminum hydroxide to absorb dy
quantitatively, with the formation of colored complexes called
These lakes when developed to their maximum intensity by pH
trol, are compared as to color intensity with standard lakes pr
from aluminum solutions of known content. As little as 0.0005
aluminum may be estimated by this method. The spectrog
method is even more delicate as a test for the presence or abse
aluminum. The great difficulty with all such determinations
prevention of contamination of the ash with aluminum durin
ashing process. The air in an ordinary room contains considerable
ing material, as is evidenced by the visibility of a beam of light f
through the air when viewed at right angles to the beam. All ord
dust contains a high percentage of aluminum. The usual practi
ashing a sample for hours at relatively low temperatures expose
dust long enough to contaminate it with quite appreciable am
of aluminum, and the metal from this source appears in the resul

McCollum, Rask and Becker ('28) employed the spectrogr
method and concluded that uncontaminated plant and animal t
contained not more than 0.5 parts of aluminum per million. Ka
berg and Closs ('29) likewise employed the spectrographic metho
found easily detectable amounts of aluminum in all samples exam
McCollum, Rask and Becker pointed out that Kahlenberg and
had mistaken two calcium lines for aluminum lines and that
spectrograms did not reveal the presence of aluminum in their
ucts. This cause of the dispute, which is still going on, is due to f
technic, usually accidental introduction of aluminum into the sa
Certainly the amounts of the element in natural products (the
portions not exposed to dirt) are almost vanishingly small.

McCollum, Rask and Becker ('28, l.c.) studied the possible ro
aluminum in animal physiology. They concluded that aluminum
pounds are not absorbed from the stomach or intestinal tract
present in the diet. Aluminum compounds when present in th
mentary tract do not form any union or compound with the sto
or intestinal walls. Aluminum in the diet in concentrations as hi
600 p.p.m. exerts no noticeably deleterious action on growth, repr
tion, or general well-being as judged by external appearance

topsy. Similar observations have been made by a number of other vestigators using the rat as the experimental animal as well as the g, rabbit, pig, and guinea pig.

The absorption of aluminum is exceedingly small. No systemic armacologic effects can be ascribed directly to absorbed aluminum. has been demonstrated that the absorption of phosphorus from the gestive tract is governed by dietary aluminum. However, this can be own only under grossly exaggerated circumstances. For example, de-essed phosphorus absorption was observed in an experiment in which 00 p.p.m. of aluminum was added to the diet. Under these condi-ns very severe rickets was produced in young animals due to the hibition or phosphate assimilation. This exaggerated condition of uminum concentration with reference to phosphorus can never be countered with the aluminum naturally present in the diet or de-ed from aluminum utensils.

That aluminum is not toxic is indicated by the fact that when it as injected instead of taken by mouth, it produced no toxic effects. these animals growth was not retarded, reproduction and lactation ere not impaired; there was no evidence of gross or microscopic thology in the organs.

All the experimental data lead to the conclusion that no harmful ects can be expected from soluble aluminum occurring naturally in ods or introduced by utensils into a diet of normal phosphorus con-nt. It is highly questionable, therefore, whether aluminum has any ysiological significance.

In 1907 President Roosevelt appointed the Referee Board of Con-ting Scientific Experts to examine into the alleged health hazard of uminum. The Board reported in 1914 the conclusion that aluminum mpounds, when used in the form of baking powders in foods, were t found to affect injuriously their nutritive value. This referred to addition of baking powder in the preparation of foods. Aluminum mpounds (baking powder containing sodium aluminum sulfate) en added to foods in large quantities (up to 200 mg. per day) may oduce mild catharsis; larger quantities usually produce catharsis. is action, the Board recognized, was due to the sodium sulfate pro-ced in the chemical reaction of the baking powder. Sodium sulfate is modern name for Glauber's salt, which has for centuries been used some extent as a cathartic drug. The Board reported that "The minum itself has not been found to exert any deleterious action in-ious to health beyond the production of occasional colic when very ge amounts have been ingested," and "When aluminum compounds mixed or packed with a food the quality or strength of said food not been found to be thereby reduced, lowered, or injuriously

affected." The Board membership included some of the most fa physiologists and eminent medical men of America.

The above investigation was instituted because of the susp that baking powder containing sodium aluminum sulfate as its constituent might be detrimental to health. Fear has been creat the public mind largely through the advertising of competing ba powders not containing aluminum. With the advent of alum cooking utensils the trade war has been largely shifted to this We have repeatedly seen literature which was distributed to h makers, informing them that the cooking of foods in aluminum sels would cause cancer. There are numerous expressions of opi by eminent medical authorities that there is no health hazard in re to aluminum. Space limitations permit only a few references.

In an editorial in the Journal of the American Medical Associa ('26) it was stated: "First, it is not at all certain that there has an alarming increase in the incidence of cancer. Secondly, it is sonable to believe that the use of aluminum cooking utensils has creased because they were found to be desirable for cooking purp Thirdly, investigations made in Great Britain under the auspice the Medical Research Council indicated that cooking, even of fruits and vegetables, for long periods of time in aluminum v showed so little aluminum in the juices after cooking that it requ the most delicate chemical tests to indicate its presence. Indeed, only the fruits but the actual acids themselves were boiled in alu num ware, without the giving off of more than slight traces of alu num."

In an editorial in Hygeia ('29), published under the auspices of American Medical Association, occurs the statement: "Due to advent of aluminum cooking utensils the sale of other types of w for this purpose has been greatly injured. As a result there has b considerable propaganda during the past few years that the cook of food in aluminum was a common cause of cancer. There is scientific evidence for such statements."

In a publication of the U. S. Bureau of Standards ('27) it is stat "There is no evidence available which would indicate that alumin cooking utensils represent a potential danger to health."

Similar statements from responsible scientific men and emin medical authorities in England, Germany, and France could be cit Those wishing to pursue this subject further are referred to a rep (Bulletin No. 3, '23) of the Mellon Institute of Industrial Resear where will be found numerous abstracts from scientific publicati and many editorial comments from medical and other reputa publications. From these sources the conclusion is very strongly s ported that aluminum is not a health hazard.

Fluorine

The element fluorine has been known to occur in animal struc-
s since 1802, when Morichini first demonstrated that fumes
ch etched glass were given off when elephants' teeth were
ed with sulfuric acid. However, because of its great reactivity the
ent was not isolated until 1886.

Fluorine is widely distributed in nature, i. e., soils, rocks, and water
he form of insoluble minerals, the most important of which is
spar (CaF_2). It is present generally in very small amounts in
ts and in animal tissues. Fluorine is found chiefly in bones and
, more particularly in the enamel, in skin, cartilage, brain, blood,
striated muscle. No convincing evidence has been as yet produced
ow that it performs any useful function in animal nutrition, or
it is an essential nutrient in animal metabolism. The literature
ng with the distribution of fluorine in biological products and the
iological effects of this element has been reviewed by McClure
.

ecause of its physiological effects rather than its nutritive value,
ine demands consideration in any discussion of mineral malnutri-
Its occurrence above certain minimum concentrations in waters,
in minerals commonly used as calcium supplements, brings about
ngements of structure in function in men and in animals.

McCollum, Simmonds, Becker and Bunting ('25) first showed the
mental effects of the inclusion of fluorine (0.01 per cent of sodium
ide) in the diet of rats. Compared with normal specimens, the
s of these animals were white and more porous and lacked the
of normal bone. The rami of the mandibles were thinner and
prominent than in the controls. There was osteoporosis affect-
he alveolar process. The rats receiving fluorine developed incisors
e anterior surfaces were bleached, whereas normal rat incisors
a bright orange color on this surface. The incisors grew back-
s so that occlusion was lost, and the teeth could no longer be
ed out by attrition to maintain their cutting edges. The teeth
poorly calcified and were brittle, causing the lower incisors to
off at the gum line, whereas the upper incisors, having no
sion, overgrew into almost complete circles and in some cases
rated the roof of the mouth. The effect was interpreted as a
grade disturbance in tooth development rather than a stimulation
r-activity. The molars were well developed, the only abnormality
ent on gross inspection being abnormal whiteness and lack of the
al luster.

had for years been the practice of farmers to feed finely ground
phosphate as a phosphorus and calcium supplement to farm

rations. Such natural phosphate may contain as much as 3 cent of fluorine. Following the observation of McCollum a ciates, Tolle and Maynard ('28) at Cornell, Reed and Huffm at Michigan State College, and others, demonstrated that feed phosphate caused loss of the reddish yellow color of normal cattle, and thickening and roughening of the mandibula McClure and Mitchell ('31) did not observe any outward ch the teeth of rats fed 0.03 and 0.06 per cent of fluorine. How results of this investigation led them to the conclusion that particularly in the form of its more soluble salts, may cause osition as an abnormal constituent in the bones, as demonst an increase above normal in the bone ash of the experimen Smith and Smith ('32) demonstrated the same outward abno in rats fed 0.05 per cent of sodium fluoride, and showed that t did not differ significantly from normal in chemical composit

It had long been known that mottled enamel occurs in teeth in certain localities in many parts of the world. In hum jects mottled enamel is characterized chiefly by the presence chalky white or paper white patches distributed irregularly o surface of the teeth. The teeth may appear dead white and u Loss of normal translucency causes an unnatural glare in the Such teeth frequently are pitted and corroded, and are stru weak; when decayed they do not hold fillings well. The mott staining, of these teeth is a secondary phenomenon, some ne coming stained. The stain varies from dark brown, almost b yellow, and is usually most pronounced on the upper central i These investigators point out that the stain tends to follow the and suggest that exposure to light and air may be a factor in duction.

Black ('16) examined mottled teeth histologically and fou the intercementing substance normally present between the rods is lacking. In severe cases the enamel prisms are not we fied. The defect occurs during tooth development, since the tip first erupted, exhibits the typical white, unglazed appearance. N formed teeth never become subsequently mottled. In gene deciduous teeth are not affected, but a few mottled deciduou were observed in Indian children in Arizona. This indicat fluorine passes through the placenta with difficulty, and thos which are enameled before birth tend to be protected.

Black ('16, l.c.) and McKay ('30) were the first to attrib abnormality to something in the water supply. The problem wa by Margaret Cammack Smith and her associates ('31, '32, '3 investigated the locality of St. David, Arizona, where mottled was endemic, and found it associated with excessive consump

rine in drinking water. Simultaneously Churchill ('31) called atten- to the presence of fluorine in waters from localities affected with tled enamel. Smith and Smith ('32, l.c.) found that when the er supply contained 2.7 to 5 p.p.m., the teeth of the inhabitants e mottled in mild to moderate degree; when the water contained re than 5 p.p.m., the mottling was moderately severe to severe, the teeth showed pitting and staining.

There are localities in every country in the world and in many es of the United States in which mottled enamel has been reported ng the human population and most of the native born inhabi- s of these sections are so afflicted. The toxicity of various fluorine pounds differing widely in water solubility seems to be the same all practical purposes. Foods containing as low a concentration as o 16 p.p.m. of dry substance induce this effect. Dean and Elvove , '36, '37) studied the minimal threshold of chronic endemic dental rosis. They determined the fluorine in the domestic water supply each of a group of 10 cities, with the following results expressed parts per million: Pueblo, Colo., 0.6; Big Springs, Texas, 0.7; lins, S. C., 0.9; Monmouth, Ill., 1.7; Galesburg, Ill., 1.8; Colorado ngs, Colo., 2.5; Plainview, Tex., 2.9; Amarillo, Tex., 3.9; Conway, ., 4.0; and Lubbock, Tex., 4.4. An analysis of these data indicated uantitative relationship between the fluoride concentration and the cal manifestations of dental fluorosis. Continuing their study, they d in the water of Clovis, N. M., 2.19; Webster City, Ia., 1.56; East ine, Ill., 1.51, and Junction City, Kans., 0.67 p.p.m. They concluded their observations that amounts of fluorine not exceeding 1 n. are of no public health significance.

The teeth are by far the most sensitive to fluorine ingestion. The ity of this dental defect, which is generally restricted in its occur- e to the period of development of the permanent teeth, is closely ed to the concentration of fluorine in the water consumed. Water aining less than 1 p.p.m. of fluorine appears to be innocuous to ast 90 per cent of exposed children; water containing from 1 to 2 n. produces a chalky white enamel, the incidence being about een 40-50 per cent; concentrations of 2 p.p.m and over induce a deeply stained, and later a pitted and corroded enamel. The ence of mottled enamel among exposed children increases to 100 ent when the fluorine in the drinking water attains a concentra- of about 6 p.p.m.

side from the dental dystrophy, which is the first symptom to r, chronic fluorine toxicosis induces cellular hyperplasia of the id, retarded ossification of the connective tissue and a greater parency of the bones, anorexia, loss of weight, estrual disturbances, ased milk production, diuresis and polydipsia. Impairment of

milk production and fetal nutrition by toxic doses of fluorine m
direct result of a depressed appetite. There is a marked distu
of the osseous metabolism, resulting in great thickening and e
of the long bones and the mandibles, although the gross ch
composition of the bone may not be disturbed except for an in
content of fluorine and of magnesium and a decreased content
bon dioxide. In sheep, the bone changes have been reported as s
ing those of osteomalacia. The fluorine content of milk is not
affected by the concentration of fluorine in the ration, but in t
of the laying hen the egg is not well protected against fluor
vasion. The coagulation time of the blood of the chickens ha
observed to decrease; the Ca, P (inorganic), and hemoglobin
blood do not seem to be affected.

Detrimental effects of fluorine upon calcium and phosphoru
zation have been observed upon various species of animals, depe
upon the concentration of fluorine in the ration and probably
factors. Only rarely have fluorine dosages been observed to affe
concentrations of calcium and inorganic phosphorus in the
plasma, nor does fluorine exert its damaging effect on tooth and
by altering significantly the concentration of the enzyme promi
involved in calcification, namely, phosphatase. The bone chang
dinarily observed in fluorosis are an increased, unchanged or dec
ash content, possibly dependent on the calcium content of the
a depression of the Ca:P ratio; an increased magnesium and flu
content; a decreased content of carbon dioxide; and a decreased
ing strength. A softening of bones has also been reported, wi
increase in volume due to enlarged marrow spaces. Tooth compo
is not so readily affected by fluorine as is bone composition, alth
the changes produced are often in the same direction.

An interesting observation is that of Phillips and associates
of the similarity of fluorosis to scurvy in guinea pigs. Their evi
leads them to believe that ascorbic acid deficiency and the effe
chronic fluorine toxicosis are due to disturbances in specific pha
cellular respiration. These studies suggest that fluorine toxicosis
duces its systemic reaction through an interference with cellula
piration and that the primary point of attack is the enzymatic sy
of the body. Phillips and Chang ('34) also observed that flu
toxicity in rats causes the vitamin C potency of the anterior lobe
hypophysis and the suprarenal gland to increase.

Little attention has been given to the pathways of entran
fluorine into the organism other than through the drinking wate
food. Murray ('36) has recently shown that fluorine can pass thr
the placenta and gain entrance to the fetus. He found that litte
rats receiving 0.05 per cent sodium fluoride in their diet, killed w

hours after birth, contained five times the fluorine content of the ntrol animals in their body ash. In further experiments the relative fluence of contact with fluorine during gestation and the consumption of milk from mothers ingesting fluoride was determined by bone alyses. The results show that the young absorb fluorine when it is ministered both before birth and after. There is not only placental nsmission of fluorine but also excretion through the milk. This indicates that mottling of the temporary teeth may result from antertum maternal fluorosis and that in regions of endemic fluorosis lk may contain biologically effective amounts of this element. These sults have been confirmed by other investigators (Knouff et al.,).

Removal of Fluorine from Water.—Fink and Lindsay ('36) und that activated alumina is very effective in removing fluorine m water at pH 7.5. Elvove ('37) found that this element may be ectively removed from water by means of calcium triphosphate, gnesium oxide, or magnesium hydroxide.

Sharpless ('36) reported that aluminum chloride inhibits to a large ent the toxic effect of fluorine. He also studied the effect of calcium ts on fluorine toxicosis. These salts had a partial protective action. found that a mixture of 2.0 per cent calcium carbonate and 2.0 cent aluminum chloride protected rats from fluorine poisoning.

Nelson ('36) has obtained similar results using aluminum sulfate.

Dispensability of Fluorine.—Sharpless and McCollum ('33) attpted to determine whether fluorine is an indispensable element in diet. They prepared a diet containing no determinable amount of rine, which was fed to young rats, starting at about 50 gm. weight. eir method permitted estimation of a range of values with differes of but 0.01 mg. of fluorine, and added amounts of this order ld be accurately estimated.

The bones of rats on their stock diet contained nearly 0.04 per cent fluorine, whereas those from rats fed the fluorine-low diet gave ues of 0.001 to 0.0046 per cent. The highest value of bones from k animals was 0.08 per cent. The addition of 1 mg. of fluorine per gm. of the fluorine-low diet caused the content of this element he bones of the animals receiving this diet to rise as high or higher n that of bones of the stock rats. They found somewhat less fluorine the teeth of rats than in the bones, and no fluorine could be ded in the teeth of the low fluorine rats. The teeth appeared to be ellent in respect to calcification, and there was no indication of es.

Since rats grew normally on the fluorine-low diet, and reproduction s not adversely affected, although the range of the fluorine content he bones was reduced to from 6 to 25 p.p.m., and was eliminated

from the teeth so far as could be determined by the method was concluded that fluorine plays no physiological role and necessary in the diet.

Selenium

Although there is reason to believe (Beath, '35, '37; Byers, and Trelease, '38) that selenium may be necessary for the d ment of certain plants, it appears to be the only mineral e known to be absorbed from the soil by plants in sufficient qu to render them poisonous to animals and perhaps to man.

The injury is greatest in livestock because they are genera solely on home-grown products, whereas the human population secures its breadstuffs almost entirely from cereals grown in parts of the country. Likewise much of the canned and other foods are obtained from afar. Consequently, the intake of sel tends to be considerably smaller in man than in farm animals.

The first scientific report of selenium poisoning appears to hav written in 1856 by Madison, a veterinarian of South Dakota, w served the condition in cavalry horses. He suggested a toxic fac the local forage as the probable cause of the disease. The occu of the disease was reported at various times in different parts country. The true nature of this disease did not become gen known until Franke of the South Dakota Agricultural College de strated the toxic character of some of the grains grown in sectio South Dakota where the disease in livestock has been more o prevalent. Following this, Robinson ('33) and Franke ('34, demonstrated the presence of selenium in the grain which had ously been found toxic to animals. Selenium is present in co trations ranging from mere traces to as high as 0.1 to 0.2 per ce a wide variety of plants growing upon certain types of soils. vegetation may induce a slow chronic disease commonly know "alkali disease" and "blind staggers," or it may be quickly fatal. disease, prevalent in the North Central Great Plains of the U States, causes considerable losses of cattle, horses, pigs, sheep, chickens as the result of ingestion of the selenium-bearing vege growing in those regions. Plants heavily contaminated with sele have an offensive odor and ordinarily are not well liked by live It is thought that any vegetation containing 5 p.p.m. of seleniu potentially dangerous although this appears to be a conservative mate. This element occurs in greatest concentration in the leav intermediate concentration in the stems, and in least concentrati the seeds. The selenium in plants is derived from and is in conc tion proportional to the selenium occurring in the soil.

Whether animals are fed with inorganic selenium compoun

th the natural plant toxicant, the pathologic and symptomatic fea-
es resemble closely those encountered among animals in the toxic
as. Cattle, horses, sheep, and pigs show erosions of the ends of the
ight-bearing bones, with hoof abnormalities that considerably im-
r locomotion. For example, cattle, after losing their hoofs, may
lk on their fore-knees. Malaise, anorexia, stunted growth, loss of
dy hair, loss of weight, emaciation, impairment of reproductive
wer, and severe anemia develop followed by exhaustion, toxemia,
d death. Poultry subsisting largely on seleniferous grains do not
ow well, are nervous, and have ruffled feathers. Chickens show a de-
ed impairment of fertility; egg production is delayed and reduced;
eggs have low hatchability. The embryos are generally deformed in
se eggs which fail to hatch. They may have short upper beaks and
ir eyes may be absent. If the eggs hatch the young do not live
g, and monstrosities frequently occur among them. Similiar mon-
sities are produced in chicks by the injection of selenium salts into
tile eggs. The symptoms encountered experimentally in the guinea
and the albino rat do not differ essentially from those in other
mals. Even if removed from the selenium-containing diet and fed
ood ration, selenized animals do not usually recover. The stunting
growth in calves is pronounced and continued, and in cattle the
nage to the kidneys and liver seems to be permanent. Hogs seem
be more easily poisoned by selenium than are Herbivora. Franke
4, l.c.) noted that rats fed on toxic grains for as short a period as
days showed a lack of recovery, growth disturbances, and typical
hologic changes even when subsequently fed a control diet for as
g as 165 days. It is of interest that the investigations have shown
t the experimental animal is able to differentiate between diets of
ying toxicity, and although extreme inanition is observed, Franke
Potter ('35) have found that the pathology is caused by the toxi-
t while the inanition is due to a voluntary restriction of food in-
e. Young animals seem to be more susceptible than older ones.
ese observations confirm those in the case of range animals.

The absorption of selenium by plants has been studied chiefly by
rd-Karrer ('33, '34, '35). Various plants show widely differing abili-
to take up selenium from a given soil. The plants which normally
orb large quantities of sulfur were found to contain large amounts
selenium. The addition of elemental sulfur to a soil containing
nium decreases the damage to the plant as well as the amount of
nium absorbed. Hence treatment of selenium soils with sulfates to
uce the toxicity of selenium is indicated.

Selenium disease at present appears to be essentially an agricultural
veterinary problem. Owing to the low content of selenium in the
ater portion of commercial crops and the high dilution of toxic

grains in the general market the danger to public health does to be great. So far human selenium poisoning has not been in the toxic areas. In the selenium regions, however, analyses vealed the presence of selenium in food, such as milk, eg wheat, barley, rye, string beans, lettuce, cabbage, and turnip amounts varying from 0.3 to 150 p.p.m. These findings in ad the results of the survey conducted by Smith and Franke ('3 rural population of parts of Wyoming, South Dakota, and N where definite proof of absorption of selenium by some of dividuals and an exhibition of a symptomatology resembling th tion in animals was noted, indicate the gravity of this problem locally and also indicate that there is no reason to believe that physiologically immune.

The researches on the cause of the animal disease now kn be induced by selenium represent one of the most elaborate an taking studies in the field of nutrition. Several bureaus of th Department of Agriculture and the Experiment Stations of So kota and of Wyoming participated in the investigation. Moxo has written the most elaborate account of these studies.

The development of the selenium problem emphasizes the recognized importance of "trace" inorganic elements in pla hence, in animal and human nutrition.

REFERENCES

Richards (1930). Biochem. J., *24,* 1572.
Peterson and Skinner (1931). J. Nutr., *4,* 419.
Orent and McCollum (1931). J. Biol. Chem., *92,* 651.
Kemmerer, Elvehjem and Hart (1931). J. Biol. Chem., *92,* 623
Orent and McCollum (1932). J. Biol. Chem., *98,* 101.
Waddell, Steenbock and Hart (1931). J. Nutr., *4,* 53.
Skinner, Van Donk and Steenbock (1932). Am. J. Physiol., *101,*
Van Donk, Steenbock and Hart (1933). Am. J. Physiol. *103,* 46
Keil, Keil and Nelson (1934). Am. J. Physiol., *108,* 215.
Daniels and Everson (1935). J. Nutr., *9,* 191.
Tutt (1934). Vet. J., *90,* 355.
Wilgus, Norris and Heuser (1937). J. Nutr., *14,* 155.
Hunter and Funk (1930). Proc. 22nd Ann. Meet., Poult. Sci. Asso donald College, Que., p. 45.
Payne (1930). Science, *71,* 664.
Titus (1932). Poultry Sci., *11,* 117.
Lyons and Insko (1937). Science, *86,* 328; Ky. Agr. Exper. Sta No. 371.
Gallup and Norris (1938). Science, *87,* 18.
Skinner, Peterson and Steenbock (1931). J. Biol. Chem., *90,* 65.

YONS, INSKO and MARTIN (1938). Poultry Sci., *17*, 12.
VERSON and DANIELS (1934). J. Nutr., *8*, 497.
ECKER and MCCOLLUM (1938). Proc. Soc. Exper. Biol. & Med., *38*, 740.
NCH and KINNISON (1933). Univ. Ariz. Agr. Exper. Sta. Tech. Bull., No. 47.
ERTRAND and BERZON (1922). Comp. rend., *175*, 289.
UBBELL and MENDEL (1927). J. Biol. Chem., *75*, 567.
EWELL and MCCOLLUM (1933). J. Nutr., *6*, 289.
ODD, ELVEHJEM and HART (1934). Am. J. Physiol., *107*, 146.
IRN, ELVEHJEM and HART (1935). J. Biol. Chem., *109*, 347.
OVE, ELVEHJEM and HART (1937). Am. J. Physiol., *119*, 768.
OTT (1934). Biochem. J., *28*, 1592.
AXWELL (1934). Am. J. Physiol., *110*, 458.
CHARGUE (1927). Ind. Eng. Chem., *19*, 1.
OX and RAMAGE (1930). Nature, *126*, 682.
ARE and ELVEHJEM (1933). J. Biol. Chem., *99*, 473.
UMBERG and RASK (1933). J. Nutr., *6*, 285.
ALTNER and WALTNER (1929). Klin. Wchnschr., *8*, 313.
RTEN, UNDERHILL, MUGRAGE and LEWIS (1932). J. Biol. Chem., *96*, 11; Proc. Soc. Exper. Biol. & Med., *29*, 174.
RTEN, UNDERHILL, MUGRAGE and LEWIS (1933). J. Biol. Chem., *99*, 457, 465.
RRON and BARRON (1936). Proc. Soc. Exper. Biol. & Med., *35*, 407.
NDERWOOD and FILMER (1935). Austral. Vet. J., *11*, 89.
ARSTON (1935). Counc. Sci. Indust. Res. Austral., *8*, 111.
NES (1935). Counc. Sci. Indust. Res. Austral., *8*, 117.
KEW and DIXON (1936). New Zealand J. Sci. Technol., *18*, 73, 688.
KEW and DIXON (1937). New Zealand J. Sci. Technol., *19*, 317.
XON (1936). New Zealand J. Sci. Technol., *17*, 600.
NHAM (1937). Science, *85*, 382.
AL and AHMANN (1937). J. Dairy Sci., *20*, 741.
WELL and MCCOLLUM (1931). Invest. Rep. No. 5, vol. 1, Bureau of Fisheries, U. S. Dept. Commerce.
RTRAND and MACHEBOEUF (1925). *Compt. rend., 180*, 1993.
TOIT and ZBINDEN (1930). Compt. rend., *190*, 172.
HER and SCOTT (1935). Biochem. J., *29*, 1055.
ATHERWICK and SAHYUN (1927). Am. J. Physiol., *81*, 560.
RTRAND and AGULHON (1913). Bull. soc. chim., *13*, 389.
IGHT and PAPISH (1929). Science, *69*, 78.
EA (1935). J. Nutr., *10*, 351
COLLUM and YUE (1937). Unpublished data.
TRAND (1920). Bull. soc. sci. hyg. alimentaire, *8*, 49.
KO (1934). Compt. rend. soc. biol., *116*, 48.
ANAMI (1932). J. Biochem., *15*, 205.
ANAMI (1933). J. Biochem., *18*, 417.
NHARDT and UCKO (1926). Biochem. Ztschr., *170*, 459.
DEK and BIER (1931). Biochem. Ztschr., *241*, 491.
DEK and BIER (1932). Klin. Wchnschr., *11*, 633, 759, 760.

Zondek and Bier (1933). Klin. Wchnschr., *12*, 55, 1411.
Jacobson (1935). Presse med., *43*, 452.
Serbescu and Buttu (1934). Bull. acad. med., *11*, 232.
Dixon (1935). Biochem. J., *29*, 86.
Neufeld (1936). Can. J. Res., *B 14*, 160.
Tanino (1931a). Biochem. Ztschr., *241*, 392.
Tanino (1931b). Klin. Wochnschr., *10*, 1046.
Neufeld (1937). Can. J. Res., *B 15*, 132.
Winnek and Smith (1937). J. Biol. Chem., *119*, 93.
Schulz (1901). Pflüger's Arch., *84*, 67.
Gonnerman (1918). Biochem. Ztschr., *88*, 401.
Gonnerman (1920). Ztschr. f. physiol. Chem., *111*, 32.
Drea (1934). J. Nutr., *8*, 229.
Brown (1927). J. Biol. Chem., *75*, 789.
King, Stantial and Dolan (1933). Biochem. J., *27*, 1003, 1007.
Stoeltzner (1908). Biochem. Ztschr., *12*, 118.
Lehnerdt (1910). Beitr. z. path. Anat. u. z. alleg. Path., *47*, 215.
Wheeler (1919). New York Agr. Exper. Sta. Bull., No. 468.
Shipley, Park, McCollum, Simmonds and Kinney (1922). B
Hopkins Hosp., *33*, 216.
Kinney and McCollum (1923). J. Pharmacol. & Exper. Therap.
Lesné, Vagliano and Christou (1924). Bull. soc. hyg. aliment
Lyonnet, Martz and Martin (1899). Lyon. med., *90*, 289.
Daniel and Lillie (1938). U. S. Pub. Health Reports, *53*, 765.
McCollum, Rask and Becker (1928). J. Biol. Chem., *77*, 753.
McCollum (1930). J. Biol. Chem., *85*, 779.
Kahlenberg and Closs (1929). J. Biol. Chem., *83*, 261, 783.
Editorial (1926). J. Am. Med. Assoc., *87*, 1397.
Editorial (1929). Hygeia, *7*, 1025.
U. S. Bureau of Standards (1927). Circular No. 346.
Report from Mellon Institute of Industrial Research (1923). Bullet
McClure (1933). Physiol. Rev., *13*, 277.
McCollum, Simmonds, Becker and Bunting (1925). J. Biol. C
553.
Tolle and Maynard (1928). Am. Soc. Animal Production, Pro
Reed and Huffman (1928). Mich. Agr. Exper. Sta. Quart. Bull.
152.
McClure and Mitchell (1931). J. Biol. Chem., *90*, 297.
Smith and Smith (1932). Arizona Agr. Exper. Sta. Tech. Bull.
213.
Black (1916). Dental Cosmos, *58*, 132.
McKay (1930). J. Dent. Research, *10*, 561.
Smith, Lantz and Smith (1931). Ariz. Agr. Exper. Sta. Tech. B
32, 253.
Smith and Lantz (1933). Ariz. Agr. Exper. Sta. Tech. Bull. No.
Churchill (1931). Ind. & Eng. Chem., *23*, 996.
Dean and Elvove (1935). U. S. Pub. Health Reports, *50*, 1719.
Dean and Elvove (1936). Am. J. Pub. Health, *26*, 567.

Dean and Elvove (1936). J. Am. Med. Assoc., *107*, 269.
Dean and Elvove (1937). U. S. Pub. Health Reports, *52*, 1249.
Phillips, Stare and Elvehjem (1934). J. Biol. Chem., *106*, 41.
Phillips and Chang (1934). J. Biol. Chem., *105*, 405.
Murray (1936). J. Physiol., *87*, 388.
Knouff, Edwards, Preston and Kitchin (1936). J. Dent. Research, *15*, 291.
Link and Lindsay (1936). Ind. & Eng. Chem., *28*, 947.
Elvove (1937). U. S. Pub. Health Reports, *52*, 1308.
Sharpless (1936). Proc. Soc. Exper. Biol. & Med., *34*, 562.
Nelson (1936). (Cited by Sharpless, 1936).
Sharpless and McCollum (1933). J. Nutr., *6*, 163.
Heath (1935). Wyo. Agr. Exper. Sta. Bull., No. 206.
Heath (1937). Wyo. Agr. Exper. Sta. Bull., No. 221.
Byers (1935). U. S. Dept. Agr. Tech. Bull., No. 482.
Miller and Byers (1937). J. Agric. Research, *55*, 59.
Trelease and Trelease (1938). Science, *87*, 70.
Robinson (1933). J. Assoc. Off. Agric. Chem., *16*, 423.
Franke (1934). J. Nutr., *8*, 597, 609.
Franke (1935). J. Nutr., *10*, 223, 233.
Franke and Potter (1935). J. Nutr., *10*, 213.
Hurd-Karrer (1933). Science, *78*, 560.
Hurd-Karrer (1934). J. Agric. Research, *49*, 357.
Hurd-Karrer (1935). J. Agric. Research, *50*, 413.
Smith and Franke (1936). U. S. Pub. Health Reports, *51*, 1495.
Woxon (1937). South Dakota Agr. Exper. Sta. Bull., No. 311.

CHAPTER

XII

Chemical Nature of Vitamin A

EARLY OBSERVATIONS.—From very early times peculiar v were assigned to certain foods for specific purposes, and investi have learned that it is wise to note all such observations, and to te validity of any and all suggestions. Hippocrates is quoted as ad ing an abundance of liver as a remedy for night blindness. M experience abundantly proves that liver is curative in this cond Among the people of Newfoundland night blindness has been mon for many years. It is reported that only men suffer from trouble, which results from the effect of the glare of sunlight on eyes while engaged in fishing. Natives of Newfoundland have believed that eating the liver of the codfish, or seagull, promptly night blindness. The efficacy of this popular remedy has been established.

Magendie appears to have been the first student of those nut principles now known as vitamins. He restricted animals to si diets composed of sugar, olive oil, wheat gluten, starch, etc., an scribed an eye disturbance now known as xerophthalmia. Living ('05) in 1857 described this eye condition as occurring among his ants in Africa, and stated that "The eyes became affected as i case of animals fed pure gluten or starch." He was apparently fa with the work of Magendie. Spicer (1892) observed sloughing o cornea of nurslings of mothers in Russia, who had practiced lon ligious fasts. Xerophthalmia was described in 1865 among slav Brazil by Gama Lobo (Pillat, '29). An historical resumé of the occurrences of the symptoms of vitamin A deficiency will be fou a paper by Eusterman and Wilbur ('32).

Before 1913 it was generally believed that all fats had similar n tive values, since they were regarded as sources of energy only,

ce their digestibility and caloric values differ but little. In that year Collum and Davis, and Osborne and Mendel, employing diets comed of more or less purified food substances, observed that rats could grow or remain in good health when lard, olive oil, or almond oil vided the sole source of fat, whereas their condition was much imved when these fats were replaced by butter fat, cod liver oil, or yolk fat. These experiments clearly indicated that certain fats ried an essential nutrient which was absent from others. McCollum ignated this factor "fat-soluble A," and it is now known as vitan A.

Steenbock ('19) conceived the brilliant idea, on the basis of such erimental data as was then available, that the vitamin A effect in ds was associated with the yellow pigment in these foods. He preed carotene, the principal yellow pigment of carrots and of many er yellow vegetables, and tested it on the rat for its vitamin A vity. From his results he concluded that carotene, but not xanphyll, afforded the same protection to rats as did butter fat, cod r oil, and egg yolk fat. Carotene is a hydrocarbon, the empirical mula for which is $C_{40}H_{56}$. It derived its name from its having been t isolated from carrots.

A disturbing fact which warranted skepticism as to the correctness Steenbock's view was the observation that cod liver oil, which is a n source of this substance, is only faintly yellow. Students of nutrin were, therefore, not convinced, but were inclined to the view that specimen of carotene employed by Steenbock was not pure but car- the vitamin A as an impurity. Several investigators at once set ut testing the validity of his conclusion, and failed to confirm his ilts. Since knowledge concerning the essentials of an adequate diet 1919 was incomplete, it is not surprising that experimenters were able, when feeding diets composed largely of purified foodstuffs, maintain growth and health in young animals even when the new soluble vitamin was provided, owing to the inadvertent omission unsuspected nutrients from the food. The failures to confirm the ritional value of carotene at that time are now easily understood, were then very puzzling.

The vicissitudes of the nutritional investigator are well illustrated he experience of Palmer and Kempster ('19), who reported an exrdinarily interesting experiment with chickens, designed to demonte whether or not yellow pigment was necessary in the diet. They ned a diet consisting of white maize, white summer squash, and te onions, which they fed to white leghorn chicks. The chicks d not grow on this food. Palmer was an expert on carotenoid pigts, of which more than 20 related substances were known, and given considerable attention to the study of their distribution. He

had observed that pig liver contained no appreciable amount o pigment. Hence, it seemed safe to include with the white v foods a small amount of pig liver. This combination prov factory for the growth and health of the chicks, and they maturity in a normal manner, except that they lacked the yel ment normally found in the skin, beak, ear-lobes, and scale shanks, which in chickens is always abundant when they are fe farm conditions. These experimental birds were thoroughly b A flock of pullets produced some 800 eggs while confined to th less diet. The yolks of these eggs were free from yellow pigm they were fertilized and hatched pale chicks, which were, reared without yellow pigment on the experimental diet. Paln Kempster's conclusion that Steenbock's opinion was erroneous fully warranted.

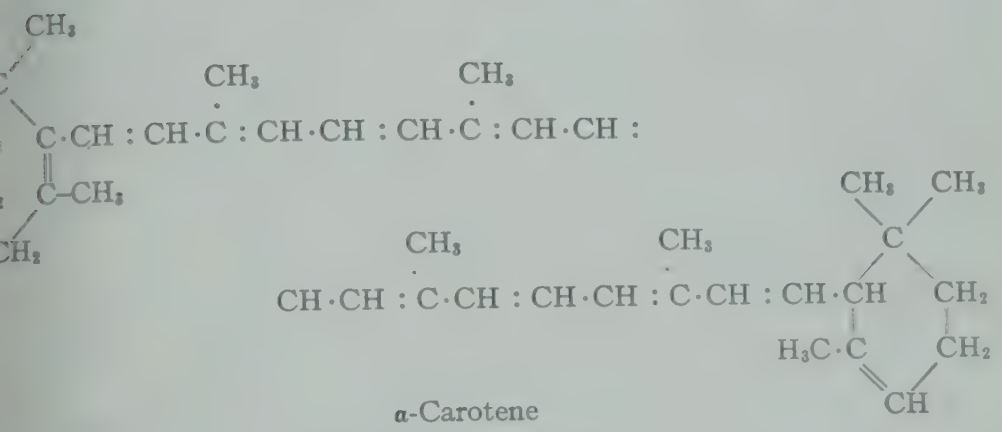
Von Euler, Euler and Hellstrom ('28) confirmed in princi experiment of Steenbock, using carotene as a source of vitamin 1922 McCollum, Simmonds, Shipley and Park demonstrated th ence of another fat-soluble vitamin (vitamin D), which is e for the normal development of the skeletal system. This substan been omitted from the diet which Steenbock had employed, s had not yet come to light, and this omission was sufficient to stunting and early failure of his rats, even when vitamin A wa plied. Von Euler and associates improved their experimental diet cluding this factor. They easily demonstrated that carotene w deed, a source of vitamin A. Their findings explained why workers had failed to get satisfactory results with carotene.

It later emerged that the reasons for the success of Palme Kempster with their birds were two-fold. The pigs which fur liver for their study had taken carotene as their source of vitan but their livers had converted the yellow pigment into the vi which is not yellow. They had kept their birds in sunlight whic ing on their feathers and exposed parts, especially the combs an lobes, had formed vitamin D. Chickens generally eat some fe and these birds probably secured some vitamin D from this s They also absorbed some from the exposed skin. Had they kep birds out of sunlight or under glass, which absorbs ultraviolet ra chicks would not have grown and different conclusions would been drawn from the experiment.

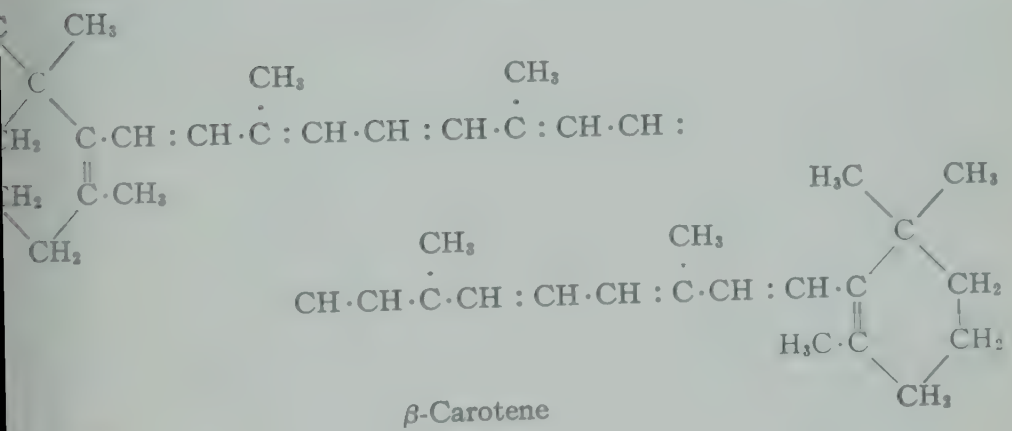
The high vitamin A value of nearly colorless fish live suggested that carotene was not itself the vitamin. The proo furnished by the studies of Moore ('29), who showed that ca is provitamin A, and is converted into the vitamin in the Moore fed highly purified carotene, shown to be devoid of vitan to A-depleted rats, whose livers thereupon became rich in vitan

was demonstrated by (a) the absence of yellow pigmentation; the blue color given by vitamin A with antimony trichloride ent (absorption band 610-630 mμ); and (c) the presence of an rption band in the region of 325 mμ. Capper ('31) demonstrated the chicken, like the rat, converts carotene to vitamin A. The oil of rats or birds fed liberal amounts of carotene, is much richer itamin A than is cod liver oil.

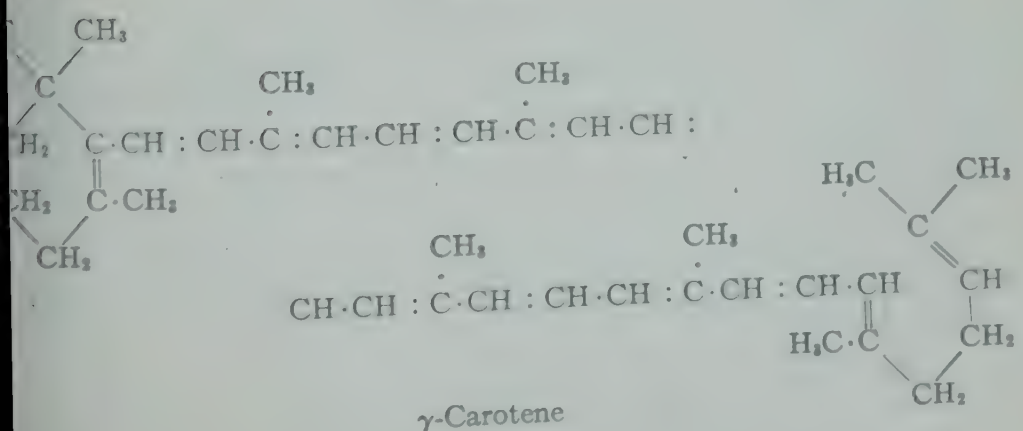
Carotene.—Carotene exists in nature in three forms, α-carotene, rotene, and γ-carotene. The structural formulae for these, and y other carotenoids, have been determined. Some of these are:



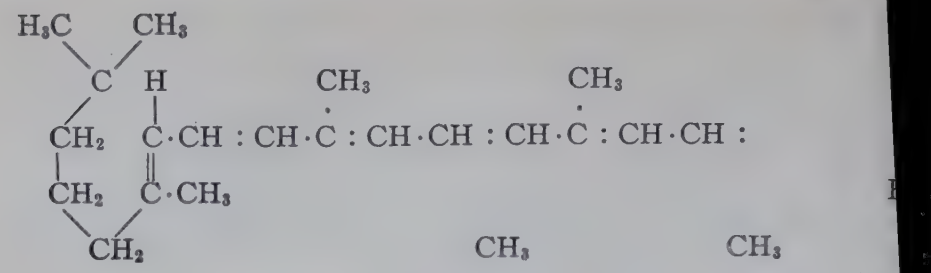
α-Carotene



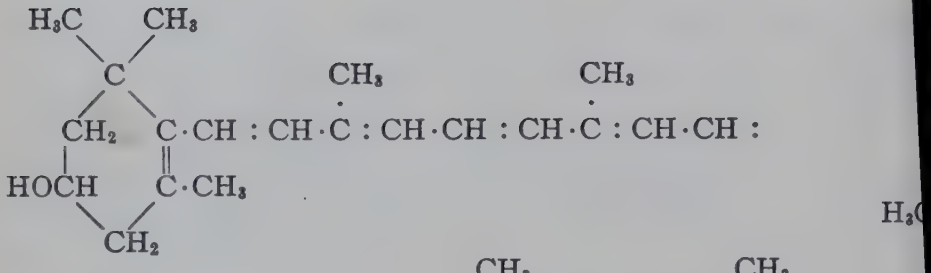
β-Carotene



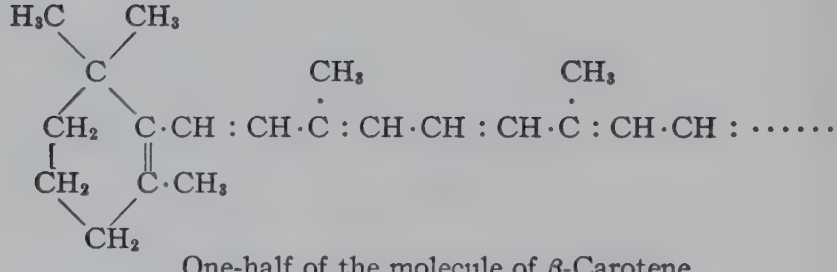
γ-Carotene



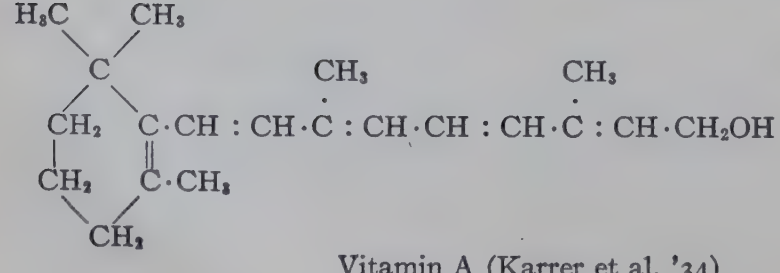
Lycopene



Cryptoxanthine



One-half of the molecule of β-Carotene



Vitamin A (Karrer et al. '34)

The carotenoid pigments occur widely distributed in plant and nimal tissues. They belong to the class of hydrocarbons known as erpenes, of which rubber is a member. These hydrocarbons are all ormed in plants from isoprene:

$$CH_2 : C \cdot CH : CH_2$$
$$\cdot$$
$$CH_3$$

Isoprene readily polymerizes to rubber under the influence of ertain catalysts. The formation of the highly unsaturated chain char-cteristic of the carotenoid pigments may be visualized as taking place hrough the polymerization of isoprene residues:

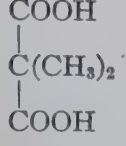
$$\cdots : CH \cdot C : CH \cdot CH : \; : \; : CH \cdot C : CH \cdot CH : \; : \; : CH \cdot C : CH \cdot CH : \cdots$$
$$\quad\quad\quad

Conversion of Carotene to Vitamin A.—Vitamin A r
formed in the liver by the cleavage of β-carotene and the addi
water to each severed end of the molecule to form an alcohol gr
shown above. β-carotene yields two molecules of the vitamin, w
α- and γ-carotenes yield but one each. The only other know
otenoid pigment which can yield the vitamin is cryptoxanthine,
occurs in yellow maize and paprika, and certain other
vegetables.

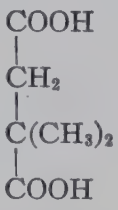
Cryptoxanthine, as shown by its structural formula, is capa
yielding on cleavage, one molecule of vitamin A, as are o
γ-carotenes. Carotenediiodide, $C_{40}H_{56}I_2$, and dihydrocarotene, a
provitamins. Dihydrocarotene, $C_{40}H_{58}$, is a yellow oil which
from reduction of carotene by means of aluminum amalgam. D
carotene results from the action of iodine on carotene dissolv
ether. It is a dark violet powder, having no definite melting po

Although it is certain that vitamin A is formed from carote
the liver, the mechanism is not well known. Olcott and McCann
employing the ultraviolet absorption test, reported the presen
liver of a carotenase which converts carotene into vitamin A. A
('31), on the other hand, claimed that carotene is not changed
vitamin A by incubation *in vitro* with liver tissue or by the a
of intestinal bacteria. However, it is well established that certain n
organisms synthesize carotene. Von Euler observed that hen's s
can transform carotene into a substance similar to but not spe
scopically identical with vitamin A. Since the liver has an impo
role in the conversion of carotene into vitamin A, it is interesting
liver oil may contain 100,000 times as much vitamin as the storage

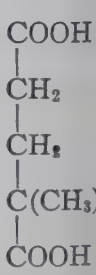
Chemical Behavior of Carotene.—On treatment with hydrog
the presence of a suitable catalyst, carotene absorbs 22 atoms of h
gen, showing the presence of 11 double bonds. Treatment
potassium permanganate yields dimethyl malonic acid, dim
succinic acid, and αα-dimethyl glutaric acid, as well as 4.5 mole
of acetic acid. Oxidation with ozone yields geronic acid. Oxid
with chromic acid yields six molecules of acetic acid. These subst
are the oxidation products of β-ionene.



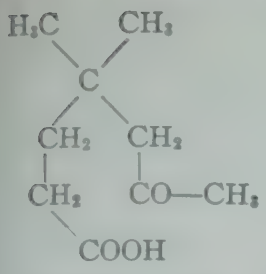
Dimethyl
malonic acid



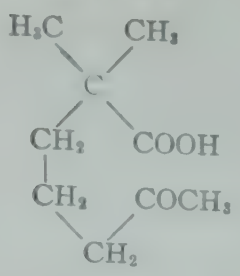
αα-Dimethyl
succinic acid



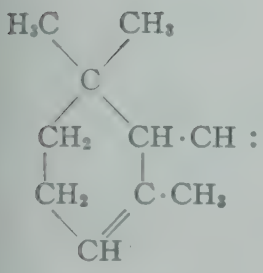
αα-Dimet
glutaric a



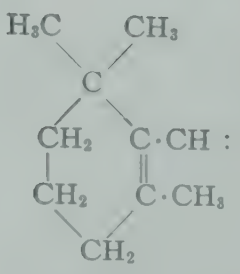
Isogeronic Acid



Geronic Acid



α-Ionone Ring



β-Ionone Ring

oxidation of α-carotene with ozone neither geronic nor isogeronic d is obtained. Quantitative experiments with β-ionone and of arotene have shown that the amount of geronic acid produced responds to two ionone-carbon rings in the latter.

Cholesterol and other sterols, squalene, carotenes, vitamin A and ious carotenoids yield blue products when treated with antimony hloride. The position of the absorption bands serves to identify the ticular substance present.

Olcovitch (Olcott) and Mattill ('31) claim that carotene is a pro-dant. Six-hundredths per cent dissolved in an auto-oxidizable oil rtens its induction period by one-half. They suggest the possibility t this property may be associated with the biological function of min A.

Properties of Carotene.—Both β- and γ-carotenes are optically ctive, whereas α-carotene in benzol has a specific rotation with mium light of +385°. When dissolved in carbon disulfide arotene shows maximal absorption of light at 521 and 485.5 mμ; arotene shows maxima at 509 and 477 mμ. The melting points of β- and γ-carotenes are respectively, 183°, 183° and 174°. γ-carotene stitutes about one-thousandth part of the mixed carotenes prepared n various plant sources. Kuhn and his associates estimated the arotene content of preparations of carotene from different sources. se relations are shown in the following table:

α-Carotene Content of Different Carotene Preparations

From Green Leaves		From Other Materials	
	per cent		*p*
Palm oil	30–40	Carrot	
Banana	25	Mountain ash berries	
Stinging nettle	0	Giant squash	
Spinach	0	Paprika	
Grass	0	Ovary	

The carotenes are soluble in acetone, petroleum ether, and organic solvents, but are insoluble in water.

The carotenes are yellow. With antimony trichloride β-car gives a greenish-blue reaction product which shows maximum ab tion at 590 mμ.

Isolation of Vitamin A.—In 1925, Takahashi and others claim have isolated pure vitamin A. This claim was later challenge Drummond ('25, '29), who expressed the belief that "the active stance is present in liver oil concentrates in amounts so minute direct attempts at its isolation by the ordinary chemical method of little use."

In 1933 Carr and Jewell, as well as Karrer and his assoc succeeded in preparing rich vitamin A concentrates by diff methods. However, in the latter half of 1937 the isolation and ide cation of crystalline vitamin A from the liver oils of three different species of fish, *Stereolepis ishinagi,* Atlantic mackerel, oil No. 1364 (an oil furnished by the Atlantic Coast Fisheries C pany) was reported by Holmes and Corbet.

Properties of Crystalline Vitamin A.—The vitamin crystal at very low temperatures from methanol, forming clusters of yellow needles. It is optically inactive and isotropic. The crystals at 7.5-8.0° C. The molecular weight, 294, and content of ca 83.28%, and hydrogen 10.44%, correspond closely to the sugg Karrer formula (molecular weight 286; 83.84% C; 10.56% H_2). extinction coefficient $E^{1\%}_{1\ cm} = 2100$ is equivalent to a mole extinction coefficient of 60,000.

In contrast with β-carotene, vitamin A gives with antimony chloride a vivid blue reaction product with maximum absorptio 610 to 630 mμ. In the ultraviolet, vitamin A shows an absor band at 328 mμ.

The vitamin has the general properties of an alcohol and este

e vitamin have been obtained. However, vitamin A is not precipi-
ted by digitonin, thus providing a means of separation from
olesterol which also occurs in the non-saponifiable fraction of animal
ts. By means of such solvents as ether, chloroform, ethyl acetate, or
ght petroleum ether, the unsaponifiable fraction can be extracted
om saponified fats without any loss of the vitamin.

Vitamin A distills in a high vacuum with partial decomposition.
ickman ('36, '37) has described a procedure for separating vitamins
and D from oils containing the vitamins in which they are subjected
an extremely high vacuum. Not only do the free vitamins distill in
ll-defined fractions under proper conditions, but the esters of the
amin, as well, behave the same in this respect. By this technique
ckman has shown that in fish oils some vitamins A and D occur
the free alcohols, most of which are esterified with fatty acids, and
t most of the fatty acids are represented in the vitamin A esters
esent. Vitamin A is reducible by aluminum amalgam in ether.

It is adsorbed by Lloyd's reagent and by norite (a charcoal). Silica
adsorbs the vitamin so tenaciously that in such form it is unavail-
e to rats, although it may be extracted from the gel with toluene.
In the absence of air, vitamin A is quite stable at high temperatures,
when oxygen is present it is readily destroyed. Vitamin A appears
be less resistant to oxidation when removed from its natural
vironment, due, no doubt, to the protective action of antioxidants.
rtain phenolic substances like hydroquinone are effective in delaying
destruction of vitamin A. Dann ('32) states that vitamin A is
ticularly stable to oxidation in ethyl alcohol, alcoholic potash,
yl acetate, n-butyl alcohol, cetyl alcohol, and triacetin. It is readily
dized by aeration in acetic, caproic, lauric, stearic, and oleic acids,
l fairly rapidly in solution in tributyrin, triolein, coconut oil, and
nut oil. It is very stable toward hydrogen peroxide when both the
amin and the hydrogen peroxide are dissolved in alcohol. Vitamin
s relatively stable to heat in the absence of oxidizing agents. Dunn
4) reported that vitamin A of cod liver oil was destroyed by mixing
oil with granulated starch and storing it in dark, corked bottles.
Collum, Simmonds and Becker ('27) found that contact of cod
r oil or butter fat with ferrous sulfate caused rapid destruction of
min A. Ferric citrate did not catalyze its oxidation, and the
on of ferrous sulfate was inhibited by an antioxidant in wheat
m oil.

Light exerts a destructive influence upon vitamin A so that extreme
tion must be used in the dispensing and storing of it. Ultraviolet
diation also destroys the vitamin.

A biological assay of the crystalline vitamin A made by Dutcher

and Guerrant (cited by Holmes and Corbet, '37) indicates a
of "considerably above 2,265,000 and somewhat below 3,400,00
national Units per gram." A similar assay by Emmet (cited by
and Corbet, '37) indicates that the vitamin "probably has a v
3,000,000 International Units per gram."

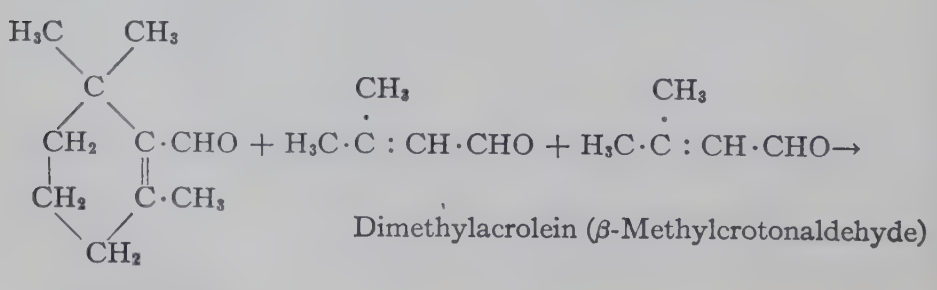
Synthesis of Vitamin A.—The animal body is dependent
food ingested for its source of vitamin A since animal tissues
synthesize the vitamin. Fruits and vegetables are practically
of active vitamin A. The carotenoid substances are synthesi
plants, and from them animals derive their supplies of this n
which is converted to vitamin A in the animal organism.

In plant tissues vitamin A exists as the provitamin, carot
may be formed in the complete absence of light, but the synth
the provitamin in plants is accelerated by light radiations, esp
of short wave-lengths. However, the ultimate amount formed
increased. This synthesis appears to parallel chlorophyll form
for green outer leaves are better sources of the vitamin than the
center leaves. Chlorophyll does not possess the biological proper
vitamin A. In plants provitamin A seems to be less distinctly asso
with the fat, and Pryde ('28) suggests that this may be due
fact that in the plant it is associated with other molecules whic
not fat-soluble.

It has been reported that certain unicellular marine organism
able to synthesize vitamin A. These algae serve as the main s
of vitamins for deep-sea animals, and the concentrations of vitam
attained by them are much higher than those found in land sc
of vitamin A.

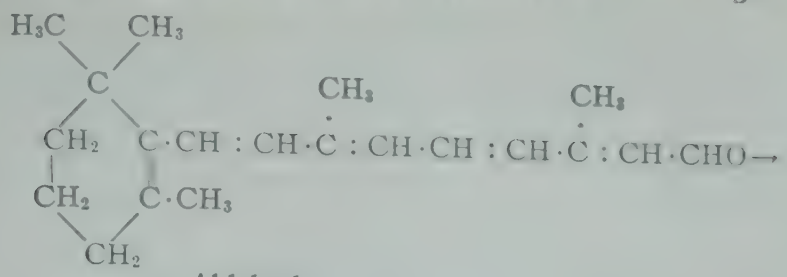
Although it has been definitely proved that certain microorga
synthesize carotene (Forster, '31), there is no evidence at this
that vitamin A is formed under the same conditions.

Two groups of workers have reported the chemical synthes
vitamin A. Fuson and Christ ('36) have condensed two molecul
dimethylacrolein with one molecule of β-cyclocitral to form an
hyde which, when reduced by the action of aluminum isoprop
yields an alcohol.

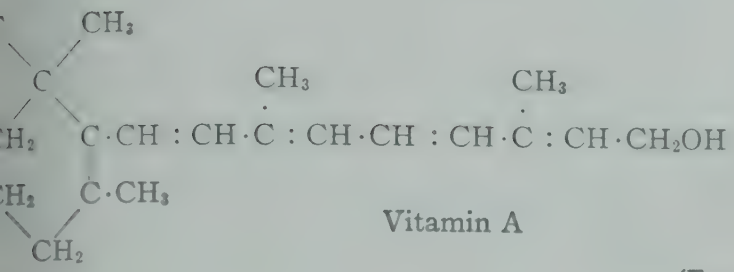


β-Cyclocitral

Dimethylacrolein (β-Methylcrotonaldehyde)



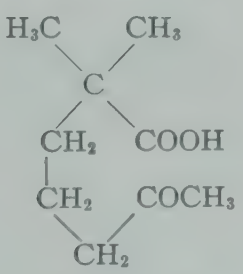
Aldehyde



Vitamin A

(Fuson and Christ—'36).

Although the resulting product has not been synthesized in pure m, it has been obtained in sufficient purity to show analytical data o its content of carbon and hydrogen, its five double linkages, its ohol group, and oxidation products corresponding to the structural mula of vitamin A. Like β-carotene, it yields on oxidation geronic

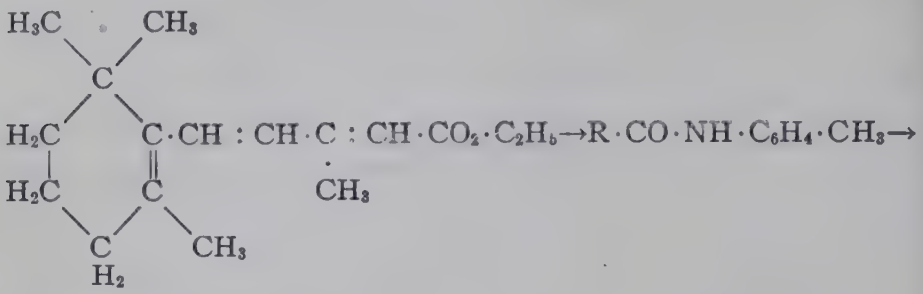


l and three molecules of acetic acid. This synthesized alcohol reacts h antimony trichloride, forming the characteristic blue color of the ndard test for vitamin A. The ultraviolet spectrum shows maxi- m absorption in the region of 328 mμ. Biological tests for the min A activity of this preparation have not been reported by these estigators.

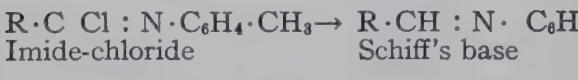
Kuhn and Morris ('37) also succeeded in synthesizing this vitamin condensing β-ionylidene acetaldehyde with β-methyl crotonalde- le in the presence of piperidine, and reducing the resulting aldehyde h aluminium isopropylate in isopropyl alcohol solution.

The β-ionylidene acetaldehyde used as the starting product was thesized from β-ionylidene acetic ester by letting this act on the gnard product of magnesium methyl iodide and o-toluidine. The luidide was treated with phosphorus pentachloride, and the imide-

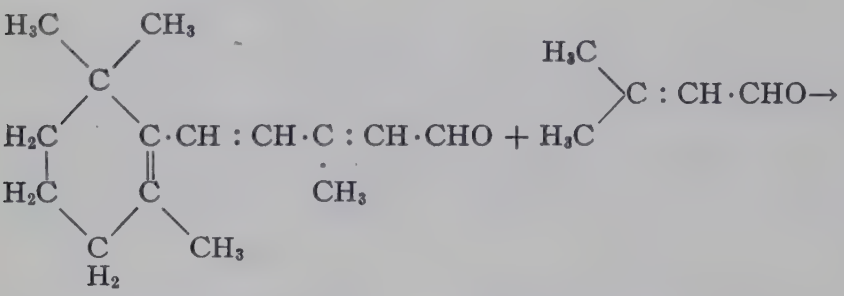
chloride was converted by the action of chromous chloride a
quent hydrolysis with oxalic acid to the free aldehyde.



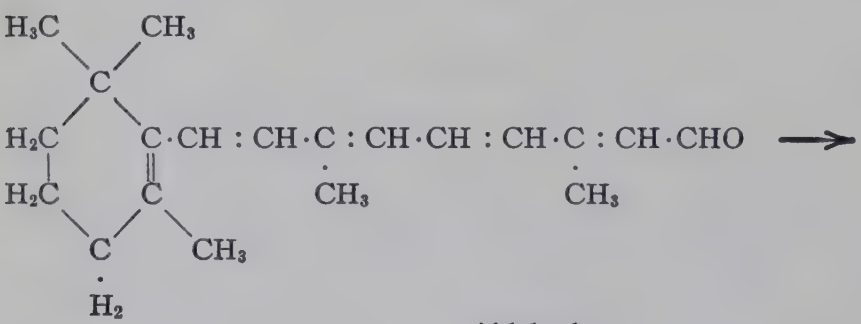
β-Ionylidene acetic ester — o-Toluidide



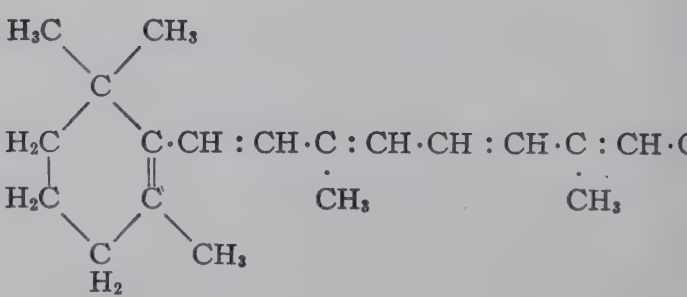
Imide-chloride — Schiff's base



β-Ionylidene acetaldehyde — β-Methylcrotonaldehyde



Aldehyde

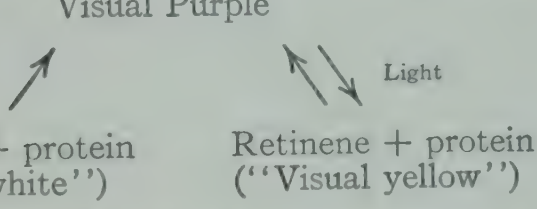


Vitamin A — (Kuhn and Morri

physico-chemical properties of their product correspond to those ribed above, as well as to the vitamin obtained from natural ces. In addition, they report the biological activity of their prepara- Vitamin A depleted rats were fed the synthetic preparation at s varying from 0.80γ to 8γ * per rat per day. Good growth was ined by the 1γ dose. With the 0.8γ dose an average gain of 11.7 in 35 days was obtained, which corresponds to the growth activity .9γ of the vitamin A standard. The 0.1γ dose had no effect on growth of rats. These results are in agreement with the activity tamin A from natural sources.

Vitamin A and the Visual Mechanism.—Vitamin A is essential ne normal behavior of the retinal organ of which it is a chemical ponent. The processes by which the visual purple and other nents in the eye are elaborated from vitamin A have been studied Wald ('35) and by Hecht and his associates ('36). Vitamin A is sported to the retina by the circulation. There it combines with protein to form visual purple which, as a result of a photo-chemical tion, is then converted into visual yellow. The latter breaks up n, producing vitamin A along with other products. Some of the min A probably is destroyed, since the continuous provision of the min in the diet is essential.

Synthesis of Visual Purple (Wald–1936)



studies of Wald showed that the retina contains a carotenoid nent, derived from the visual yellow other than carotene or min A, which he named retinene. Wald explains the process of l response to light as follows: When light strikes the visual le in the retinal cones the former is bleached to an orange pigment d visual yellow. During this process retinene, which is yellow in , is formed. The yellow color gradually fades and the retina mes colorless. When this stage is reached the retinene seems to been converted into vitamin A. In the normal living animal the min A is resynthesized into visual purple. This cycle appears to ntinuous. However, unless the eye continuously receives a supply tamin A through the circulation, the regeneration is incomplete. is the first proven example of a vitamin being an actual structural onent of a tissue.

* One millionth of a gram.

A Possible Vitamin A_2.—Recent investigations (Gillam, et '38; Edisbury, et al., '37) describe the detection of a substance in the pigment epithelium of the eye and in the livers, liver c concentrates from fresh-water fishes, yielding with antimo chloride an absorption band at 683-696 mμ. It is believed th product may have for fresh-water fishes the same physic functions as vitamin A has for marine fishes. The evidence sidered sufficient for proposing that this chromogen shall b visionally called vitamin A_2. It is homologous with vitamin A rhodopsin system. Its retinal precursor might correspondin designated retinene$_2$. The prophyropsin system is tentatively lated:

$$\text{porphyropsin} \overset{\text{light}}{\rightleftharpoons} \text{retinene}_2 + \longrightarrow \text{vitamin } A_2 + \longrightarrow \text{porphyro}$$

(Wald, '38.)

The International Unit of Vitamin A.—In 1931 an Interna Conference of nutrition experts met, under auspices of the Leag Nations, and adopted standard units for the best known o vitamins. Samples of pure carotene, prepared by a number o chemists in different countries, were sent to The Institute for M Research in London, where they were pooled, recrystallized, biologically tested. One gamma (one-millionth of a gram) o sample was adopted as the International Unit of vitamin A. Fu research showed that this sample was a mixture of β-carotene α-carotene. The biological value of one milligram of the mixture equivalent to 0.6 mg. of pure β-carotene. Accordingly, in 1934, β-carotene was substituted for the International Standard of Refer Small samples of the β-carotene in coconut oil as a reference stan are available to nutrition workers in many countries. Also a secon reference material has been prepared. This is a sample of cod oil which has been carefully compared by animal experiments the carotene standard.

The unit of vitamin A activity is, therefore, defined as the am of activity contained in 0.6γ of the International Standar β-carotene.

Methods for Estimating Vitamin A in Biological Produc Three methods,—chemical, physical, and biological—for determi vitamin A activity are available. Although the chemical and op methods offer advantages of speed and reproducibility, the biolo method is fundamentally more accurate and reliable. At presen color reaction is known to be specific for vitamin A activity w correlated with the biological method.

The chemical test, using a Lovibond tintometer or similar in ment, measures the intensity of blue color developed by the anhyd

imony trichloride solution in chloroform. This test is only roughly proximate, but it is of some value as an indicator of the vitamin A tent in oils of known history, preliminary to their biological assay. Since vitamin A is characterized by selective absorption in the raviolet, the intensity of this band, whose maximum is at 328 mμ, ves as a measure of vitamin A content. The spectrophotometric cedures are of limited applications, because expensive equipment necessary and their specificity is questionable in oils of unknown gin and also because the carotenes and allied substances of known min A activity fail to absorb at this wave length. However, adaptas of this method have been useful in industrial laboratories in the ing of fish liver oils where the vitamin A activity is due entirely he vitamin.

Although biological assays are subject to variations due to uncontable factors in the animals, at present they afford the most reliable ans for estimating the physiological activity of the vitamin. In the ogical procedures a diet is employed which is complete in all ects except for the absence of the vitamin. The basal diet is studied animals which are given a supplement of the source of vitamin he results being interpreted on the basis of a particular criterion. methods which have been studied most thoroughly are the owing:

The *Curative Method* is based upon depletion of young rats of vitamin A reserves, and then observing the rate of growth onse which occurs when a known supplement of a substance is ided which contains vitamin A or a provitamin A. Sherman has posed a unit quantity which is the amount necessary to produce a vth response of 3 grams per week in young rats under specified ditions. This method does not call for a control series of experits in which the same conditions are adhered to, and in which a rence standard cod liver oil of known potency is fed. The conion factor of Sherman units into International Units is generally d to be about 1.4. Owing to variation in response of individual nals and of rats from different colonies, this method is not very ble in the hands of different workers. Experiments run in parallel ne tests on the unknown substances should always be conducted a standard reference oil. Failure to do this has resulted in the arance in the literature of many untrustworthy assay values. The nin A test diet is described in the U. S. Pharmacopoeia XI ised 1937), and a thorough presentation of recommended experital details to be observed will be found there, as well as in ard's ('38) "Biological Standardisation of the Vitamins."

The *Prophylactic Method* is based upon the same type of experi as is the curative method, but in this case the smallest dose of

the substance under assay is determined which will just pre
appearance of symptoms of vitamin A deficiency.

In addition, a method based on the changes in the vaginal
of animals has been described as a criterion for the determin
vitamin A. This method has not proved satisfactory on a qua
basis, for although the response of the rat to large doses of the
is well marked, it is difficult to estimate slight responses to sma

Distribution. Data regarding the distribution and con
vitamin A in foodstuffs and certain other products is extensi
quantitative distribution of this vitamin is presented in the Ap

REFERENCES

Livingstone (1905). "Travels and Researches in South Africa." Harper and Bros., New York.
Spicer (1892). Lancet, *2*, 1387.
Pillat (1929). Arch. Ophth., *2*, 256 and 399.
Eusterman and Wilbur (1932). J. Am. Med. Assoc., *98*, 2054.
McCollum and Davis (1913). J. Biol. Chem., *15*, 167.
Osborne and Mendel (1913). J. Biol. Chem., *15*, 311.
Steenbock (1919). Science, *50*, 352.
Palmer and Kempster (1919). J. Biol. Chem., *39*, 299, 313, 331.
v. Euler, Euler and Hellstrom (1928). Biochem. Ztschr., *203*, 3
McCollum, Simmonds, Shipley and Park (1922). J. Biol. Chem.,
Moore (1929). Biochem. J., *23*, 803, 1267; (1930). Biochem J., 2
(1931). Biochem. J., *25*, 275.
Capper (1931). Biochem. J., *25*, 265.
Karrer (1933). Oppenheim's "Handbuch der Biochemie der Me und der Thiere" 2 Ed. Ergenzungsswerk, I, 63, 79, Jena.
Karrer (1934). Chem. Rev., *14*, 17.
Olcott and McCann (1931). J. Biol. Chem., *94*, 185.
Ahmad (1931). Biochem J., *25*, 1195.
Olcovitch and Mattill (1931). Proc. Soc. Exper. Biol. & Med., 2
Takahashi, Nakamiya, Kawakami and Kitasato (1925). Sci. Pape Phys. Chem. Research, *3*, 8.
Drummond, Channon and Coward (1925). Biochem. J., *19*, 1047.
Drummond and Baker (1929). Biochem. J., *23*, 274.
Carr and Jewell (1933). Nature, *131*, 92.
Holmes and Corbet (1937). J. Am. Chem. Soc., *59*, 2042.
Hickman (1936). Nature, *138*, 881.
Hickman (1937). Ind. Eng. & Chem., *29*, 968.
Dann (1932). Biochem. J., *26*, 666.
Dunn (1924). J. Biol. Chem., *61*, 129.
McCollum, Simmonds and Becker (1927). J. Am. Med. Assoc., *88*
Pryde (1928). "Recent Advances in Biochemistry." Churchill, Lond

STER (1931). Biochem. Ztschr., *236*, 276.
ON and CHRIST (1936). Science, *84*, 294.
IN and MORRIS (1937). Ber. deutsch. chem. Gesellsch. (B), *70*, 853.
LD (1935). J. Gen. Physiol., *18*, 905.
LD (1935). J. Gen. Physiol., *19*, 351.
LD (1938). Proc. Am. Physiol. Soc., p. 210.
LAM, HEILBRON, LEDERER and ROSANOVA (1937). Nature, *140*, 233.
LAM, HEILBRON, JONES and LEDERER (1938). Biochem. J., *32*, 405.
SBURY, MORTON and SIMPKINS (1937). Nature, *140*, 234.
CHT, CHASE, SHALER and HAIG (1936). Science, *84*, 331.
VARD (1938). "The Biological Standardisation of the Vitamins." Billiere, Tindall & Cox, London.

CHAPTER

XIII

Nutritional Significance of Vitamin A

THE TWO EARLIEST effects of experimental vitamin A defi to be observed were cessation of growth and ulceration of the c of the eyes. Goldschmidt ('15) observed keratomalacia in the e rats on Hopkins' multiple deficiency diet. McCollum and Simr ('17) described the eye condition resulting from more specif ficiency of vitamin A, as xerophthalmia, a condition which had previously spoken of by several investigators as "fat deficiency dis Mori ('22) described keratinization and desquamation of kerati epithelium of the cornea, and conjunctival and lacrymal epithelium. Daniels, Armstrong and Hutton ('23) observed tha depleted of vitamin A die of bacterial invasion of the ear and cavities before the appearance of xerophthalmia. Following these observations, numerous studies have been made of the physiolog pathology of avitaminosis A. The results of this deficienc essentially the same in experimental animals as in man.

Since the significance of vitamin A in relation to nutrition health is revealed by the effects of the absence of this nutrient the diet, a review of the extensive experimental nutritional and ological studies supplemented by the clinical observations available form the basis for the consideration of this subject.

General Effects of Vitamin A Deficiency.—A variety of symp follows the depletion of vitamin A in the animal organism. Dif animals of the same species under the same general conditions become affected quite differently. Species variations in symptomat are also quite marked. In young animals interruption of grow one of the earliest signs of a lack of vitamin A in the diet. The a becomes emaciated. Its fur is dry and coarse, and the feet are r and scaly. These are, however, common to several types of defici

nsequently, they cannot be considered specific evidences of avita-
nosis A.

It is believed that in both adults and older children hemeralopia is
arly always the earliest manifestation of vitamin A deficiency
randsen, '35; Sweet and K'Ang, '35). In infants and small children
s symptom is frequently overlooked. Hemeralopia may, for a num-
of years, be the only sign of the deficiency in adults (Frandsen, '35,
; Spence, '31). Even severe degrees of this condition can exist
thout demonstrable changes being present in the eyes.

As the avitaminosis progresses, gross anatomical changes in the
s are observed. Patchy dryness, areas of thickened epithelium, loss
luster, and wrinkling of the conjunctiva are all considered to
aracterize the second stage of the disease. There is also decreased
retion of tears. At this time signs of vitamin A deficiency are noticed
other parts of the body. Keratomalacia is the last stage of the eye
nifestations. The corneae are softened, permitting the invasion of
teria, and often blindness results.

Skin lesions, both in experimental animals and in man, are also
ly indications of the disease. Nicholls ('33, '34) and Frazier and
('36) found that dryness of the skin preceded the eye changes by
eral weeks. Bloch ('31) and others have called attention to the dry,
y, shriveled condition of the skin of infants suffering from de-
ency of vitamin A. Pillat ('29) and Sweet and K'Ang ('35, l.c.)
e that the skin undergoes more marked and extensive changes than
other tissues excepting the eye. The sebaceous and sweat
nds, hair, and nails are affected. Frazier and Hu ('31) first pointed
that in this deficiency the most specific lesions, keratotic papules of
ying size, appear, distributed especially over the thighs, arms, and
ulders, which arise from the pilosebaceous follicles. These workers
eve that age is a determining factor in the appearance of the skin
ons. The eruption is said to be rare in children, but rather common
dults, and when fully developed probably represents late or severe
ciency. MacKay ('34) and Frazier and Hu ('36, l.c.) believe that
lesions may exist before xerosis is present, and are a valuable
of vitamin A deficiency, particularly when the presence of
eralopia cannot be ascertained, as for instance in the case of infants
young children or in cases where adults cannot be tested. These
ervations have been confirmed by other investigators (Loewenthal,
Goodwin, '34). Sheer and Keil ('34) point out that a complication
es in assessing the effects of A deficiency on the skin because
ules due to vitamin C deficiency occur in early and mild cases, and
mble very closely those seen in avitaminosis A. They suggest the
bined use of the capillary resistance test and biopsy observations
keratinization in the skin follicles. Youmans ('37) has successfully

treated cases with papular lesions by cod liver oil. Critic studies on human subjects are needed to clear up the dis recorded in the literature. The type of skin lesions described i has not been observed in rats. Skin lesions in rats appear animals are over four months of age. They are in the form ears and tails, sores on the nose, sore feet, and ragged hair.

In the latter stages of the deficiency, priapism is frequ served. By this time abscess formation often takes place in t glands near the base of the tongue in the pharynx, and frequently have hematuria.

Another experimental clinical feature is the strikingly cidence of calculi and calcareous deposits in the renal pass urinary bladder in rats long deprived of vitamin A. Less symptoms are muscular incoordination and altered sensitivity

These characteristic symptoms are not limited to the r have been observed to occur in a variety of other species, i man, when there is a shortage of vitamin A in the diet.

In contrast to the many experimental studies of vitami ficiency, clinical investigations have been few, and such as are deal mostly with infants and children. It is but a short time symptoms of this condition in the adult have begun to be lo clinically.

Visual Disturbances.—After the discovery of vitamin A, gators for several years depended upon cessation of growth appearance of xerophthalmia as signs of deficiency of this nu experimental animals. The xerosis of the conjunctiva sugges sible vitamin A deficiency in human subjects afflicted with this In 1863 Bitot (cited by Blegvad, '24) had described xerosis tivae in 29 patients, all of whom suffered from night blindn thought the xerotic covering of the eye cut off some of the li thus caused impaired vision in subdued light. In the same yea (cited by Blegvad, '24) asserted that he had observed night b in patients who did not exhibit xerosis. Eventually ophthalm reached the conclusion that night blindness, xerosis conjuncti keratomalacia represent different degrees of the same ailme Gouvea (1883) described night blindness in malnourished s Brazil and noted that they were unable to see when returni work after sunset, but could see well when starting for wor sunrise, although it was much darker in the morning than evening. This is a common experience of persons who suff night blindness caused by deficiency of vitamin A. This d vision follows exposure of the eyes to light. Night blindness due to other causes such as the congenital type, and the type retinitis pigmentosa and to detachment of the retina, alcohol

ori ('04), Bloch ('17), McCollum ('22) and others have compiled
rlier references to night blindness associated with malnutrition, and
e use of liver and cod liver oil as remedies for this ailment.
hthalmologists have, for obvious reasons, been confused as to the
ology of night blindness. In general, they have agreed that exposure
bright light may cause the disease, and that resting the eyes in
rkness may cause remission, while exposure to light may cause
apse.

A new concept was thrown upon this problem by the investigations
Fridericia and Holm ('25) on experimental night blindness in
mals, and the chemical observations on the regeneration of visual
rple after it has been bleached by exposure of the eyes to light.
ght blindness is due to failure of vision in faint illumination. Hess
7) had studied the effect of previous illumination on visual acuity
hens by watching them pick up grains in light regulated as to
ensity. He found that after a stay in the dark they would eat in a
ht less intense than that in which they would stop eating without
vious exposure to darkness. Holm ('25) devised a jumping test
vision of rats, making use of the dislike of the rat to remain on
surface of a table, and its desire to hide under shelter. The animals
e placed on a table, the edge of which was about 10 cm. from a
lf on which was a cage that afforded shelter. The cage was marked
h a piece of white paper to make it clearly visible. The table was
quently turned to new positions to make the rats depend on sight.
s released on the table quickly explored its edges and, when they
ld see the cage, immediately jumped over to it. The degree of
mination could be controlled. Vitamin A deficient rats were com-
ed with normal rats. In dim light the difference between normal
A depleted rats was very apparent. The former would see the
and immediately run and jump across the gap to it. The depleted
would explore the table top and perhaps stop at the right corner,
for a leap but give up the attempt. As a rule, they would at last
p, but not infrequently from the wrong corner, and fall to the
r. The attitude assumed in jumping was different from that of
mal rats. When a stronger lamp was lighted, the A depleted rats
ld see the cage and act as did the normally nourished rats.
ision of vitamin A resulted in improvement of vision.

These studies were extended by an examination of the visual
le of the retina of normal and depleted rats, and of rats whose
had been exposed to strong light. Most ophthalmologists believe
t blindness to arise from an abnormality in the rod cells of the
a, which are the only cells in which visual purple occurs. Vision
ylight, with the perception of colors, is believed to be the function
e cones, whereas the vision of twilight, in which colors are not

distinguished, is the function of the rods, and is dependent
purple. Lythgoe ('37) has pointed out that the deep sea
retinae composed almost exclusively of rods, and these a
filamentous. He suggests that visual purple, the light-se
stance, is adsorbed on their surfaces, and that the large
rods in the conger by increasing the quantity of visual
proves the animal's vision at low illuminations. He also stat
hunting monkeys have a cone type of retina, whereas ni
species have retinae composed mostly of rods.

The Visual Acuity of Dysadaptation Test.—Since th
Holm ('25, l.c.) showed clearly that vision at low illur
dependent upon the presence of visual purple in the
Fridericia and Holm ('25, l.c.) showed that the content of th
in the retina is decreased in avitaminosis A, and, further
night blindness is an early symptom of A deficiency, it wa
suspect that an examination of the ability of the eyes to ad
selves to vision in light of low intensity might be made
indicator of incipient vitamin A deficiency. This idea wa
to the test of experiment in this country by Jeans and Zentm
Using the Birch-Hirschfeld photometer they measured the
to light of the eyes of 213 children following partial dark a
Forty five were found to have subnormal dark adaptation. T
were kept under observation and were given a good diet
cod liver oil. All who were retained for study regaine
adaptation, the average time for recovery being 12 days. La
Blanchard and Zentmire ('37) described a new biophoto
this purpose, and suggested further precautions for making
With the new procedure they found 35 per cent of 23 child
orphanage to have subnormal adaptation. They concluded
I. U. of vitamin A per day meets the normal requirements of

Edmund and Clemmesen ('36-'37) used a different in
which permitted testing the visual acuity, the visual field,
visible, and power of distinction. They studied a long series
and of hemeralopic individuals. About half of their pregnan
showed low adaptation, which was severe in cases compl
hepatitis and by vomiting. In patients other than those exhi
disease or pregnancy, the incidence of low adaptation was
cent. Intramuscular injection of 1 cc. of a preparation said t
40,000 I. U. of vitamin A caused improvement which did n
beyond 7 days. Double this dose produced improvement
than one and less than two weeks.

These investigators also made quantitative studies on a
prisoners in Denmark, from which they conclude that 800
day per man gave rise to oscillations in the power of d

gh rarely dysadaptation proper. Twelve hundred and twenty I. U. day permitted oscillations in the power of distinction in a few indiuals, and in some cases dysadaptation. They believe the minimum tective daily intake of vitamin A to be about 1370 I. U.

Park ('35, '36a) found by means of the dark adaptation test, 83 cent of A deficiency among Indian children in Oklahoma. Jeghers -), who has reviewed the literature on this test, found 34 per cent young adults deficient in A. On the other hand Snelling ('36) was able to obtain convincing results with the Birch-Hirschfeld photoer. Palmer and Blumberg ('37) observed marked variability in cessive tests, and urge caution in drawing conclusions from the as it now stands. Their examinations did not indicate that Maryl and the District of Columbia school children were deficient in vitamin. Hecht ('37) has contributed an excellent review of the ature on rods, cones, and the chemical basis of vision. He points that, while the primary light reaction is probably simple, visual ple regeneration is complicated, in that it is influenced by pH, a dark after-bleaching effect. The primary dark reaction probably lves more than the direct reconversion of photoproducts into itive material, since visual purple may be formed both by regenerafrom the products of bleaching by light, and by the addition of material. At present there seems to be widespread skepticism as ne reliability of the dark adaptation technic as a measure of vitamin eficiency.

riderichsen and Edmund ('36-'37) describe a method for estimatvitamin A nutrition in infants by determining the smallest nsity of light that will produce reflex movements of the face and when the infant is adapted to complete darkness. Certain icially-fed infants were less sensitive to light than breast-fed ins, and absorption and utilization of vitamin A was so rapid after inistration of fish liver oils, that a normal light response was ined half an hour later. They point out that treatments involving iction to water, barley water, or rice water, rapidly exhaust the min A reserves.

Changes in Epithelial Tissues.—The detailed investigations by bach and Howe ('25, '28, '33), Goldblatt and Benischek ('27), many others, lead to the conclusion that vitamin A is essential nost vertebrate species, and that its absence is indicated by a mon effect on epithelial structures. They pointed out that the st observable consequence of vitamin A deficiency is atrophy of elial structures. These are replaced in many locations, especially lands and mucous membranes, by stratified keratinizing epium identical in appearance in all locations and arising from proliferation of basal cells. The mucosa of the stomach and

intestines, and the renal tubules, show practically no ch
atrophy occurs, it progresses to a state wherein the cells
serving the appearance of viability, become inert physic
well as in their function of covering cell membranes. Th
respond by active mitotic division, with the production
thelial cells as a reparative function. Regardless of previ
and morphology, the new cells continue to grow and un
original epithelium, and form into a stratified, keratinizing
In the liver and renal tubules, in addition to their p
activity, the mature epithelial cells possess the power t
These new cells do not respond as do other epithelia by ke
Why the reparative activities of basal cells of many differe
lead in vitamin A deficiency to a common product, an epi
structure, cannot be answered, they believe, unless ke
represents a reversion to a more primitive type than th
secretory activities. On the provision of vitamin A, the
characteristic of each region returns to its normal type. The
have undergone keratinization are either cast off or disinteg
ification of the external layers of the skin is a normal p
takes place independently of the vitamin A supply. There
a cyclic keratinization of the vaginal epithelium which ap
under hormonal control and constitutes a phase of the estru

Wolbach ('37) points out that the striking gross pathol
dition of vitamin A deficiency in animals and in human inf
outcome of accumulation of keratinized epithelial cells
glands and their ducts, as well as in other organs. In glandu
cysts of considerable size may form, which contain yell
masses of keratinized cells. In the lungs of human being
animals, A depletion leads to cyst formation, bronchial occl
sequelae such as bronchiectasis and atelectasis. Such cysts
at the base of the tongue, and have been described as
Daniels et al. ('23) described the accumulation of cheesy
the sinuses and internal ear. Wolbach states that although
and the plugs of desquamated epithelial cells in ducts, br
trachea, being open to invasion by bacteria from without,
culture medium for those bacteria, there is very rarely invas
underlying tissues, presumably because of the protective f
the keratinized layer of epithelium.

In the rat, Wolbach observed the order of response to av
A by metaplasia to be: (1) salivary glands, and accessory
the tongue, buccal cavity and pharynx; (2) respiratory tract,
nares, maxillary sinuses, Jacobson's organ, trachea and br
genitourinary tract, including renal pelves, ureters, bla
didymis, prostate, seminal vesicles, coagulating glands, uterus

nd accessory glands of the vulva; (4) eye and parocular glands, rneal and palpebral conjunctiva and the harderian, intraorbital and traorbital lacrimal, and meibomian glands. In the guinea pig the es and related structures are refractory respecting keratinization as mpared with the rat, whereas keratinizing metaplasia in the uterus d bladder occur earlier and are more severe than in the rat.

In human infants Wolbach observed keratinizing metaplasia in the njunctiva, nose, and accessory sinuses, trachea, bronchi, pancreas, nal pelvis, ureters, salivary glands, uterus, and periurethral glands. ne commonest and earliest appearance of keratinization of epi- elium in infants is in the trachea and bronchi. Next in frequency d sequence is the kidney pelvis. In infants deficient in vitamin A, e changes in the mucosa of the respiratory tract appear to account the frequency, severity, and persistence of pneumonia. In the man subject, as in the rat, the eye lesions occur late. The cornea comes vascularized, edematous and infiltrated with leucocytes. In- tion of the cornea is favored by the accumulation of keratinized ls, and by paucity of tears for washing the eye and may lead to popyon, and ulceration of the cornea.

Manville ('37) counted the goblet, or mucous secreting cells of mparable areas in the stomach, pylorus, and large intestine, and nd a marked reduction in the number of these in vitamin deficient animals. He points out that reduction of mucous secretion poses the lining of the gastrointestinal tract to injuries caused by tion with coarse particles of food. The presence of occult blood the feces, caused by bleeding from such injuries, he believes is the liest evidence obtainable of this deficiency. It is well known that ly in A depletion there is an increase in the number of keratinized helial cells in washings from the conjunctival sac and in urinary iment. Manville points out that since the protein moiety for mucous mation is not deficient in the A free diet, paucity of mucous pro- tion may be related to lack of glycuronic acid, which is a pros- ic group of mucin, the glycoprotein of mucous. He fed animals nthol, which is conjugated in the body with glycuronic acid to uce its toxicity. When the doses exceeded the supply of glycuronic for conjugation, toxic symptoms appeared, and animals surviving a few days showed ulceration of the stomach, pylorus, small and e intestines, and gall bladder. These ulcers bore a marked re- blance to those seen in vitamin A deficient animals. It is logical onclude that impairment of function of the goblet cells would unt for the reduction of mucus secretion as well as deficiency of uronic acid as a structural unit for the formation of mucin.

Changes in the Nervous System.—Nervous symptoms in this type vitaminosis may result from alterations within the central nervous

system or in the peripheral nerves. These symptoms depend o nerves affected.

Hart and McCollum ('14), and Hart, Miller and McCollum pointed out that swine suffering from lack of vitamin A exh symptoms of muscular incoordination, which could be cured by ing butter fat. They showed that there were marked degene changes in the nervous system. Similar observations have bee ported by Steenbock, Nelson and Hart ('22), Hughes et al. ('28, Mellanby ('26), and Aberle ('34).

Rats depleted of vitamin A show no striking nervous sympt incoordination occurs as a late symptom. In the vitamin A dep rats, Aberle observed clumsiness, incoordination, and finally spas with complete loss of control of the affected limbs, but not unti weight increment was reduced to zero, or until xerophthalmia s venes late in the course of the syndrome.

Zimmerman and Cowgill ('36) recently described the lesions o nervous system produced by this deficiency. These investig found the myelin degeneration in the sciatic nerves greater tha the brachial plexus. No lesions were demonstrable in the cereb In the spinal cord, degeneration of the medullary sheaths in sensory and motor tracts was quite extensive.

In swine, muscular incoordination is, according to Hughes coworkers ('28, l.c.), the outstanding symptom, while eye sympt which are so prominent in the rat, may be absent. In swine the watering of the eyes, but no severe conjunctivitis, and keratiniz of the tissues about the eyes seems not to be pronounced. How impaired vision occurs.

One form of posterior paralysis in hogs is due to degeneratio the medullary sheaths. Guilbert, Miller and Hughes ('37) obse that the development of this paralysis is usually secondary to n blindness. Prior to this condition there is incoordination of m ments. Back muscles on one side only may become involved, resu in more or less marked scoliosis. Incoordination and spasms in sw due to A deficiency, suggest nerve involvement. The finding Hughes et al. are not contradictory to the view expressed by Wolb that nerve lesions are a late manifestation in avitaminosis A. time required to produce incoordination in swine is not stated Hughes. His work confirms earlier studies which show that in vanced A deficiency, degeneration of nerve cells occurs in the optic, sciatic, and femoral nerves. Hughes found that the ner symptoms in swine could be prevented by feeding cod liver oil, bu fat, yellow corn, or alfalfa leaf meal; hence, deficiency of water sol vitamins was presumably not involved. Vitamin A therapy, if i tuted early, results in marked improvement of general muscular

rdination, weakness and even posterior paralysis, but complete overy is rare. The lesions in the nerves once developed are usually manent and probably often progressive (Hart and Guilbert, '37). E. Mellanby ('29-'30, '31) presents a different interpretation of the rbid histology of avitaminosis A. He has given special attention the degenera.on of the gasserian ganglion and the trigeminal ve, which is distributed in the skin and structures of the face, gue, teeth, eyes, etc. He supposes that xerophthalmia is an expres- n of degenerative changes in the nerve supply of the cornea, and suggests that hyperplasia or metaplasia of the various epithelial mbranes may be related to changes in their afferent nerve supply. llanby and King ('34) suggest that the periodontal metaplasia ich occurs in avitaminosis A, and which may be the cause of rrhea, and possibly of dental caries, may be an expression of loss neurotropic control.

E. Mellanby ('34) proposes vitamin A administration in prophy- s and treatment of septicemias and of infectious diseases, and sug- s vitamin A deficiency as the cause of nerve degeneration in beri, pellagra, disseminated sclerosis, pernicious anemia, lathyrism, convulsive ergotism. He cites 13 patients with disseminated rosis, who were greatly improved by vitamin A therapy. Wolbach ises caution in acceptance of Mellanby's observations because they end upon the "capricious Marchi technic" and because degenera- of the myelin sheath is common to many disorders which result n several vitamin deficiencies and diverse causes.

That caution is indicated in drawing conclusions concerning a ific relation between avitaminosis A and nerve degeneration is gested by the studies of Zimmerman and Burack ('34), who ob- ed degenerative lesions believed to be identical with those seen human pellagra, in animals receiving sufficient vitamin A, and cient only in the "black tongue-preventive factor." Clinically, min (vitamin B_1) without A is successfully used in the treatment eriberi, in which nerve injury is the outstanding feature. Investiga- s are greatly to be desired in which an uncomplicated A deficiency udied with respect to nerve lesions and other signs of avitaminosis such as the skin manifestations, keratinization of conjunctival pings, keratinized cells in the urinary sediment, dysadaptation of n, etc. It is seldom that even the informed reader can be certain experimental diets have been complete as respects all factors other vitamin A.

Lathyrism is a condition commonly seen in India and Northern ca and occasionally in Southern Europe, which is ascribed to a otoxin in lathyrus peas. Spasticity and rigidity of the large muscles degenerative changes in the spinal cord are its chief features.

Mellanby ('30) has produced a similar condition in dogs by a d composed of a variety of pea (Akta) and deficient in vitamin Animals receiving this diet supplemented with A showed but slig degeneration of the spinal tracts. Further supposition that this vitam may furnish protection against lathyrism is supported by the expe ments of Geiger, Steenbock and Parsons ('33). They produced exp mental lathyrism in both young and adult rats by a diet of the flow ing sweet pea, itself rich in vitamin A. Young animals were not p tected against toxicity of the sweet peas by cod liver oil or cod liv oil concentrates. However, adult animals were protected to son extent.

Effects on Growth.—In the early days of vitamin research t growth promoting property of vitamin A was emphasized. A numb of investigations demonstrated that deprivation of this nutrient resu in cessation of growth and that the requirement of this vitamin f growth and health is greater in the young animal. It has also bee observed that growth varies with the amount of the vitamin in th diet and the amount stored in the tissues of animals. However, growt failure in itself is not specific evidence of vitamin A deficiency; it believed that interruption of growth resulting from lack of vitamin is a secondary result of a disturbance in the metabolism of epitheli tissues. This interruption of growth, common both to infants and t laboratory animals, is associated with loss of weight due largely t loss of fat in all storage depots, and also to atrophy of musculatur and many organs, *e. g.,* liver and kidney, which do not undergo kerati nizing metaplasia.

Changes in Bone Tissue.—Extensive changes are found in th bones in A deficiency. Tozer ('21) found that the bones of animal deprived of vitamin A were fragile and exhibited minute ridges a the rib junction. Histologically, after 14 days on the deficient die the changes in the costochondral junction were indistinguishabl from those occurring in the ribs of scorbutic animals. After 21 day of depletion, considerable reduction was observed in bone length, bot of the trabeculae and the rows of cartilage cells. These changes wer progressive and by 30-40 days the rows of cartilage cells and trabecula had almost disappeared. The cartilage often ended in a thin, ossifie band, which was apparently laid down to support a weak joint After a period of 50 days, due to the marked fragility of the bones fractures of the ribs occurred and sections showed proliferation o cartilage cells. The cartilage and bone itself are densely calcified Harris ('26) has allocated a special function to vitamin A in connec tion with the growth of bone. He considers ossification proper specific function of A.

Wolbach ('37, l.c.) has shown that in this avitaminosis there is

tion of proliferative activity of the epiphyseal cartilages. Those which are in the vesicular stage disappear, and there remains only n line of atrophic cartilage cells embedded in a calcified deposit h forms a plate of bone on the diaphyseal side. This gives sec- of immature bones an appearance similar to that of adult bones. n vitamin A is supplied, there is a prompt response by the capil- blood vessels, which, arising from the bone marrow, penetrate the deposit enclosing the vesicular cartilage cells, causing its resorp- following which active cartilage cell regeneration takes place and nal bone growth is resumed.

ffects on Teeth.—The tooth lesions in this condition, which are dered by Wolbach and Howe ('33, l.c.) to be the most important al changes of any of the deficiencies, are striking and characteristic. ameloblasts, or enamel forming organs, are of epithelial origin. aminosis A causes them to atrophy and undergo metaplasia, with equent formation of hypoplastic enamel. Mellanby ('28), who has ed the influence of vitamin deficiencies on tooth development, has n clearly that teeth formed in animals completely deprived of nin A have no enamel; while in less severe deprivation the enamel fective, of poor structure, and contains pits and fissures which ispose the teeth to decay. Similarly, normal dentin formation is ndent upon an adequate supply of vitamin A. There is no reason ubt that this vitamin plays a more important role than does any specific nutrient in maintaining the normal function of the nel-forming organs.

Wolbach and Howe ('33, l.c.) have described the changes which r in the incisor teeth of rats and guinea pigs in avitaminosis A. atrophy of the ameloblasts is accompanied by keratinization. is followed by atrophy and loss of polar deposition of dentin ix by the odontoblasts, the dentin-forming cells. The odontoblasts in morphologically normal and functionally active on the labial of the tooth in apposition to the enamel organ long after the plete disappearance upon other surfaces. With complete atrophy ne ameloblasts in the rat the odontoblasts disappear also on the l side. Although they survive, the odontoblasts lose their columnar e and continue to deposit dentin, but in a centrifugal pattern like blasts or bone-forming cells, instead of at the outer pole only n the normal cells.

ffects on the Hematopoietic System.—In addition to the inter- ion of growth, degenerative lesions of the skeletal muscles, lym- d hypoplasia of the spleen, and an anemia which is accompanied he accumulation of hemosiderin in the liver and spleen, and by phy of the spleen and bone marrow, are secondary results of the rbance in the metabolism of epithelial structures due to vitamin A

depletion. Berglund, Keefer and Yang ('29) believe that other fa appear to be necessary to produce this form of anemia. Similar servations have been reported by Keefer and Yang ('29), Pillat Yang ('30) and John ('31). Koessler et al. ('26) suggested that t is a relation between vitamin A deficiency and pernicious ane but Sure, Kik and Walker ('31) were unable to substantiate t observations.

Koessler, Maurer and Loughlin ('26, l.c.) have reported a mar decrease in the red cells and hemoglobin in A deficient rats. Th apparently is no reduction of the leucocytes in the blood stream these animals. In fact, a polymorphonuclear leucocytosis has b reported (Findlay and McLean, '25) to be not uncommon after onset of keratomalacia.

Cramer, Drew and Mottram ('22, '23) and Bedson and Zi ('23) noted a diminution in the number of blood platelets in A pleted rats independent of their growth propensities. This chan becomes markedly manifest before infective conditions appear. Cram and Drew ('23) suggest that platelets are formed by the endotheli cells of lymphatics and blood vessels. Administration of A stimulat the production of platelets and delays the development of bacteri infection. Falconer and Peachey ('26), on the other hand, find th changes in the relative number of platelets, red cells, and white cel not constant enough to constitute a specific lesion in vitamin A d ficiency.

Lesions in the leucoblastic bone marrow are rarely found in A d ficient animals, except occasionally in a very few adult animals deplete for a prolonged period. An aplastic condition of the marrow occur (Findlay and Mackenzie, '22). In the early stages of the deficiency some slight atrophy, but no hemorrhage of the bone marrow, is foun (Tozer, '21, l.c.). Later, progressive atrophy occurs, and in extrem cases little but the delicate reticulum of the connective tissue ground work and the blood vessels remains. Upon the provision of vitamin A these pathological effects are arrested and the tissues tend to retur to normal, provided infectious processes do not intervene. The addi tion of this nutrient brings about hyperplasia of the lymphoid tissue increased bone marrow, and complete blood regeneration.

Metabolic Changes.—Sure and Smith ('31) found that in variou stages of vitamin A deficiency, as indicated by the severity of th eye lesions, there were no significant changes in the concentratio of blood sugar. The carbon dioxide capacity of the blood remain normal in this condition. The content of liver glycogen in A deficien rats was normal. There was reduced food intake, and excessive wate consumption in their animals.

Synthesis of purine bodies is diminished in vitamin D depletio

rgan and Osburn, '25). According to these investigators, in the nal animal the excretion of allantoin diminishes with a gain in ght and increases as the weight falls. Exactly opposite results are ined in the case of the vitamin A deficient animals. Emmett and ock ('25) observed a disturbance of uric acid metabolism in this iency. Kimura ('28) reports that experimental hypercholestero- ia in the rabbit returns to normal slowly upon administration itamin A in the form of biosterin, Takahashi's name for vitamin A. Green ('34) observed that depletion of vitamin A in the rat pro- d a marked decrease in the esterase content of the blood serum. metabolism is not altered since fat is still absorbed, mobilized, turated, and oxidized in the absence of vitamin A. Administra- of vitamin A produces a marked rise in the serum cholesterol.

Urolithiasis.—Osborne and Mendel ('17), Van Leersum ('28), Carrison ('31), Higgins ('33), and many others have reported finding of urinary calculi in rats deficient in vitamin A. Higgins) found urinary calculi in 85 per cent of A deficient rats which ived 250 days. These stones were composed principally of calcium sphate with a trace of calcium carbonate. In studies on both human ects and on rats, he finds the sequence of events in calculus forma- to be: (1) keratinization of epithelium of the genitourinary t, (2) urinary infection, and (3) alkalinuria. The low solubility calcium phosphate in alkaline media accounts for the separation rystalline phosphate. In rats, bladder infection usually occurs after days and renal infection after 60-90 days of deprivation of vitamin Addition of ammonium chloride to the diet causes a decrease in formation of calculi, owing to its acidifying effect. Higgins asserts precipitation, per se, causes sediment and not stones. Desqua- ed epithelial cells are present in the urinary tract. Stone formation, vever, is facilitated because keratinized epithelium creates suffi- t irritation and local lesions to produce fibrin and mucin which n a frame work for the deposition of crystalline sediment and sequent development of stones.

Oppenheimer and Polack ('37) contested the claims of Higgins high vitamin A intake and acid ash diet will prevent the forma- of phosphate stones. Most investigators of this subject believe vitamin A deficiency is but one of several causes of urolithiasis. regular adherence to a diet affording an alkaline ash would ainly increase the likelihood of deposition in the kidney or bladder calcium phosphate stones because of the alkalinity of the urine, the insolubility of calcium phosphate in alkaline media. The ent day emphasis upon "alkalizing" the blood is based upon acy. Another cause of stone formation probably is hypochlorhydria. en the stomach does not secrete sufficient hydrochloric acid, the

urine tends to become and remain alkaline. This condition is rected therapeutically by the administration of acid. Infection of bladder, ureters, and kidneys, with ammonia production from u fermentation, quite apart from avitaminosis A, would presuma cause stone formation. All considerations point, however, to the pr ability that avitaminosis A does cause loss of power of the mucosa the urethra, bladder, and other areas in the genitourinary tract, prevent invasion of the tract by microorganisms which are norma present about the urethral orifice but which, in health, are unable thrive on a mucous surface. The excessive growth of fuso-spiroche organisms in the mouth of dogs restricted to a blacktongue-produci diet, demonstrated by Smith and coworkers ('37), is a case in poi Only the etiology of phosphate stones is suggested in relation avitaminosis A. From the studies of O'Conor ('35), it appears th deficiency of this vitamin is significant in promoting the formati of calcium carbonate stones, especially in subjects restricted to alkali diets for therapeutic purposes. He states that recurrence of these ston is frequently prevented by a diet rich in vitamin A.

Hou ('35), Lelesz and Przezdziecka ('35), and others, have a serted that rats depleted of vitamin A develop urinary calculi mor readily when given large doses of vitamin D. The relationship i humans between vitamin A depletion and the formation of kidne stones still remains controversial.

Disturbances of Reproductive Function.—Long and Evans ('20) described the cell changes in the vaginal mucosa during the estrua cycle of the normal rat as follows: stage 1) small, nucleated epithelia cells only; stage 2) large, cornified, non-nucleated cells; stage 3) intensification of stage 2—a great many cornified cells; stage 4) poly morphonuclear leucocytes, cornified cells and large epithelial cells o only cornified cells and leucocytes, or occasionally, large nucleated epithelial cells and cornified cells; and stage 5) polymorphonuclea leucocytes and epithelial cells. In 1922 Evans and Bishop reported a change in ovarian function in vitamin A deficiency. This change is characterized first by the appearance of abnormal numbers o cornified epithelial cells (keratinized cells) and the disappearance o leucocytes. This cornified phase becomes further and further pro longed until the entire cycle is characterized by cornified epithelia cells. If gestation ensues, animals occasionally exhibit resorption o the fetuses when depletion of the vitamin is severe. Usually they carry their young to term or slightly beyond, exhibiting an obscure impair ment of the birth mechanism which ordains fetal death. Lesser severity of vitamin A deficiency may permit rhythmic estrus, ob scured by the continuous cornified smear, but evidenced by the re currence of copulation. Even though conception does not occur, the

ual cycle is not appreciably lengthened, so there is obscure in- rement of that endocrine mechanism which establishes pseudo- gnancy after infertile copulation.

Macy and coworkers ('27), Evans ('28), and others, conclude that presence of cornified cells in the epithelial layers of the vagina he first and most delicate symptom of vitamin A exhaustion. This v is not shared by Parks and Drummond ('26) and Coward). However, recent investigations (Mason, '35) seem to establish se findings.

Aberle ('33) found that continual cornified peripheral vaginal cells eared in 100 per cent of rats on a diet deficient in vitamin A, and this symptom invariably preceded other symptoms of avitami- s A. The time elapsing was positively correlated with the amount he vitamin contained in the diet or stored in the body.

Sherwood, Brend and Roper ('36) gave rats massive doses of caro- in oil for 15 days, and observed that such doses prevented a nor- vaginal smear picture. They examined 18 rats at 8 hour intervals ugh eight complete estrus cycles. These rats were then given I. U. of vitamin A in the form of carotene daily for a period of days. A second group of 16 rats were similarly observed, and were given 3750 I. U. of carotene for 15 days. Control animals on same diet were given 0.5 cc. daily of cottonseed oil, or of corn and showed normal vaginal smears throughout the period of rvation. In the experimental animals receiving massive carotene, smears did not progress from the nucleated epithelial to the ified cell stage. There was an excess of nucleated epithelial cells rdless of the phase of the estrus cycle. This was due to a rapid growth. These results confirm those of Mason and Ellison ('35).

pson and Mason ('36) treated thirty cases of senile vaginitis in rly women who were subsisting upon poor diets, by oral adminis- ion of cod or halibut liver oil. Vaginal repair was rapid and com- e. This study seems to point the way to dietary prophylaxis against condition.

Mason ('33) has made the most critical study of the effects of erent states of nutritional deficiency on the testes of the rat. In so-called "incomplete total inanition" or the state induced by ing a diet containing all essentials, but fed in insufficient amount, n brought about after the animals have attained sexual maturity, testicular injury resembles in some respects that caused by vitamin eficiency. There occurs cellular sloughing and gradual reduction seminiferous tubule size. Depletion of germinal epithelium is r complete.

n uncomplicated A deficiency there is testicular degeneration, profound injury to the seminiferous epithelium. The residual

germ cells show evidence of continued attempts at sperm form even in tubules whose epithelium is reduced to a few layers of Injury to the seminiferous epithelium is not irreparable, as in vit E deficiency, since the restoration of an abundance of vitamin the diet leads to regeneration and complete reinstatement of the lar epithelium in a high percentage of cases. Mason states that te lar injury occurs before other external symptoms of A defici appear. He believes that testicular injury and vaginal cornifica are two early manifestations of disturbed metabolic processe epithelial tissues. It appears that the more highly organized physiologically specialized the epithelium, the more readily disturbed by lack of any factor necessary for its metabolic n Repair of avitaminosis A injury to the testes requires from 5 t weeks. During vitamin A therapy, the repair process is not gre retarded when the body weight of rats is maintained at a subnor level, or is even caused to decrease. The testicular damage afte depletion cannot be attributed to an indirect effect resulting f hypophyseal disturbance, but is one of the manifestations of the e of this deficiency on epithelial structures in general.

Moore and Mark ('36) observed, in a clinical study, metapl and inflammation of the prostate in man which resembled the eff of vitamin A deficiency. A series of vitamin A depleted rats sho atrophy of the testes and accessory sex glands which these inve gators believe to be indicative of some disturbance in the hy physeal-gonadal-prostatic hormone relationships. Avitaminosis A the rat they found to be associated with foci of inflammation a epithelial metaplasia in the prostatic acini and vesicular ducts simi to that reported in other organs. Focal metaplasia and inflammat is occasionally encountered in the prostate in cases of extreme inanit associated with stenosis of the esophagus. It seems probable that t lesion is due to vitamin A deficiency.

Relation to Infection.—The literature on the relation of vitan A deficiency to resistance to infection is very extensive and confl ing. In 1935 the Council on Pharmacy and Chemistry of the Am can Medical Association defined its attitude toward the permissi claims which advertisers may make for vitamin A in cod liver as follows: "By virtue of its vitamin A content it promotes grow and, as indicated by experimental studies, may be an aid towa the establishment of resistance of the body to infection in gene though it has not been shown to be specific in the prevention colds, influenza and other such infections." At this time the Cou has not altered its position on this subject.

Since it is generally conceded that deficiency of vitamin A impa the health and functions of epithelial membranes, the possibility t

minosis A might increase susceptibility of the organism to colds other respiratory diseases, has been tested by several investigators. ner and Gardner ('34) studied two groups of carefully paired ren from 6 to 14 years of age. The experimental group received o "units" daily of vitamin A in halibut liver oil. The control p was selected on the basis of a high resistance to colds. In the ed group there was a marked decrease in severity and incidence lds, accompanied by improvement in health in general.

ameron ('34) divided 65 women students into five groups. The bers of one group took a tablespoonful of cod liver oil daily from mber to March. The other groups were given respectively a in A concentrate, a cod liver oil concentrate in tablet form, an tablet, and no medication. The number of days with colds re- d from the group taking no medication was considerably higher from any of the other groups.

olmes et al. ('36) made a five-year study of the effect of admin- ion of cod liver oil to an industrial group on absenteeism caused olds and other respiratory disease. In the first to the fifth year sive, there were respectively, 341, 566, 691, 648, and 785 subjects r observation. Approximately half of these were given about 5 spoonfuls of cod liver oil weekly. The results indicate that colds other respiratory infections were materially lessened in the cod oil group as compared with the control group. Necessarily, ele- ts are involved in such studies which make it impossible to ap- e quantitatively figures employed in the expression of the results he observations. Eventually, sufficient data will be accumulated ake clear the general tendency of this form of dietary prophylaxis s effect on respiratory disease.

Clinical findings are conflicting. From the facts available it is ossible to evaluate these results because the intake of the vitamin not definitely established and the nutritional condition of the s studied was extremely varied. In conclusion, investigations in- e that vitamin A is anti-infective merely in the sense that it pre- s the typical keratinization of tissues produced by avitaminosis A secondary infections. Vitamin A does not effect general immunity. various experiments indicate that there is no basis for the belief vitamin A therapy is effective clinically in infections caused by ific highly pathogenic organisms. Also it is of no value in infec- s diseases which are unassociated with the characteristic structural kdown of epithelial tissues, and the accompanying localized in- on typical of vitamin A deficiency.

Relation to Artificially Induced Infections.—Verder ('29) fed eficient rats and a normal group, suspensions of *B. enteritidis* for n days, after which time the animals were killed. A greater num-

ber of the A deficient animals showed positive spleen cultures for organism than did the controls. Szulo and Kolodziejska (cited Robertson, '34) report decreased resistance in A deficient rats to human strain of tuberculosis as compared with controls.

Boynton and Bradford ('31) found that young white rats inc lated subcutaneously with a standard suspension of bacilli of *mucosis capsulatus* group, after 4, 6, 8, and 10 weeks' depletion vitamin A, showed marked decrease in resistance to this infecti as compared with the controls receiving cod liver oil.

Finkelstein ('32) found that mice depleted of vitamin A a infected with bovine tuberculosis, ran a more acute course than w the case in mice receiving the same diet plus 0.005 mg. of carote per day. McClung and Winters ('32) observed that 15 days af the intraperitoneal injection of mouse typhoid I, only about 5 p cent of their A deficient rats were alive, whereas about 95 per cent the controls which received cod liver oil were alive. As will be show in the discussion of vitamin D, this appears to have little or no effe on the course of several types of infections. These typical studies, whic are in harmony with the earlier work of Daniels ('23, l.c.) and Mellanby and Green ('29), and Green ('32), must suffice as a fa sample of the rather extensive literature on vitamin A in relation t artificially induced infections.

Effects on Immunological Reaction.—The immunological prop erties of the blood of A deficient animals appear to remain unchanged The bactericidal power is lowered, however. Findlay ('25) does no believe this to be a direct cause of vitamin A depletion, since a reduc tion of this factor occurs in all acute infections. Both Smith and Wason ('23) and Findlay and McLean ('25, l.c.) reported a perceptibly reduced phagocytic activity simultaneous with the appearance of keratomalacia. Tanaka (cited by Robertson, '34, l.c.) states that there was a reduction of agglutinins and amboceptors in rats and guinea pigs deficient in A after injection of cholera and typhoid bacilli, as compared with the controls. v. Euler ('31) found that when carotene was given in liberal amounts to rabbits the amboceptor in the blood was increased. Green ('33) restricted rabbits to a diet deficient in vitamin A and observed that there was no reduction in the natural hemolysin or complement titre of the blood. Upon injecting typhoid bacilli into normal and vitamin A deficient rabbits, she found that the deficient animals produced somewhat less antityphoid agglutinins and bacteriolysins than did the controls. When sheep's red blood cells were used as the antigen, the hemolysin production in the deficient rabbits was much below normal.

Relation to Thyroid Function.—Abelin ('33) and Wendt ('35) observed that administration of vitamin A concentrates to cases of

ophthalmic goiter reduced the basal metabolic rates. They found treatment especially suitable in cases where the continuous use odine is inadvisable. According to Wendt ('36), Abelin ('36), and and Buchanan ('37) thyroxin and vitamin A are antagonistic. y report abnormally low blood concentrations of vitamin A and, lesser degree, of carotene in cases of exophthalmic goiter. After essful iodine or surgical treatment the vitamin A and carotene es of the blood were normal. Wendt suggests that in myxedema tene is not completely converted to vitamin A. These observations been confirmed by a number of investigators. Abelin ('36, l.c.) ts out the merits of using vitamin A as auxiliary treatment in medication with iodine and with diiodotyrosine and thus lessening "crisis" and the temporary exacerbations that usually occur in forms of medication. However, more experimental data are nec- y before a definite evaluation of vitamin A therapy in hyper- oidism is possible.

Role in Healing of Wounds.—A number of clinical publications nr and Unger, '37; Chevallier and Escarras, '37; and others) report ficial effects of cod liver oil ointments in cases of skin burns and nds. Healing of wounds produced in experimental animals is erated by cod liver oil, the total unsaponifiable fraction of cod oil, by vitamins A and D in moderate concentrations, and by easily-oxidized unsaturated fatty acids, particularly as the free and in the presence of vitamin A.

Vitamin A in oily solution, applied to the surface of experimental nds, causes a marked change in the character of the exudate and oid shrinkage of the edges of the wounds.

unctions of Vitamin A.—The functions of vitamin A are not definitely established. However, the vitamin appears to be essen- for normal growth, for normal function of the eyes, and for al structure and function of the epithelial tissues. It has been ulated whether vitamin A is an integral part of structures like elial cells or whether it acts as a catalyst of certain cellular func- The symptomatology produced by avitaminosis A offers little ht into the precise function of this nutrient in the tissues.

bsorption.—Due to the close relationship between carotene and in A, and also due to the fact that the vitamin has but recently isolated and synthesized, experiments dealing directly with the in itself are not available.

he process of digestion is not a requisite for absorption and utiliza- of this nutrient, but the degree of absorption is widely different rotene and the vitamin, varying with the intake, manner of ad- tration, and the health of the alimentary tract. Both are soluble in nd fat solvents and are absorbed by the lacteals of the intestine,

thus becoming intimately associated with the chyle. They enter circulation through the thoracic duct (Drummond et al., '35). change in either substance takes place during the absorbing proc The liver plays a significant role in regulating the concentration the vitamin throughout the body. Examination of normal livers vealed that the amount of A tends to remain constant within cert limits (Hess et al., '33). When the diet is poor the vitamin A rese of the liver is less than in the normal. Experiments indicate that e complete removal of A from the diet requires a considerable per of time to deplete the liver vitamin reserve.

Assimilation of carotene is extremely variable. Under normal c cumstances carotene is somewhat less efficient than the vitamin.

Carotene when administered in small quantities, under ordina conditions, is utilized to the extent of about 80 per cent. Larger quan ties are not as well utilized as vitamin A, particularly if the carote is fed without being dissolved in oil.

One of the best illustrations of the difference in the absorpti of carotene and vitamin A is the effect of mineral oil on the abso tion of these substances. Vitamin A appears to be satisfactorily utiliz in the presence of this compound, but the absorption of carotene markedly diminished. The vitamin administered with large amoun of the oil may be largely lost to the body if the concentration of is very slight, and of the oil is very great. There is very little eviden of such loss when the oil and the vitamin are given separately. Th vitamin as well as carotene is less well absorbed in paraffin oil tha in vegetable fat and better on a fat-rich than on a fat-poor diet.

The presence of bile is not necessary for the absorption of vitami A from the gastrointestinal tract of the rat. Greaves and Schmidt ('35 showed that rats whose bile was diverted to the upper part of th descending colon by means of cannula, did not respond to oral ad ministration of carotene as did normal rats. They were responsive however, when glycodeoxycholic acid or deoxycholic acid was simul taneously given, indicating that these bile acids function as carrier of carotene across the intestinal wall. Variations in the fat content o the diet from 3 to 23 per cent did not materially influence the absorp tion of carotene from the alimentary tract. On the other hand, th vitamin itself was absorbed by rats with internal bile fistulae. Simila results obtained with jaundiced animals support the view that, wherea vitamin A can be absorbed from the intestine in the absence of bile the presence of this body fluid is essential to the utilization of caroten given by mouth.

Dietary intake alone of the vitamin is obviously not sufficient t insure its utilization. Reports of secondary cases of avitaminosis A ar appearing in the literature, which are due to deficient absorption o

s substance. Among this group are cases of gastrointestinal and patic disease, of severe infectious diseases, and of increased requirements due to rapid growth.

Excretion.—Green ('32, l.c.) believes that the urinary excretion s not account for any significant loss of vitamin A, and that if en in excess, the unstored portion of the vitamin is destroyed in liver or oxidized in the blood stream. Schneider and Weigand ') investigated the elimination of vitamin A in 180 cases. They nd that the normal organism does not eliminate it in the urine n after administration of excessively large doses. However, a large centage of patients with cancer, tuberculosis, or general infections, excrete the vitamin in urine. The authors believe this to be caused liver impairment or by a change in the renal permeability.

Vitamin A normally plays an important role in renal physiology. In aminosis A, dogs and human subjects are slower in starting and not maintain diuresis as well as normal animals.

Rowntree ('30) demonstrated in experiments with young children even after the ingestion of an excess of vitamin A only 2-12% lost in the feces. Wendt ('37) determined the vitamin A content he feces of normal subjects with and without vitamin administra- and also of patients with various disturbances in the processes of rption. The cases receiving the vitamin showed a slight increase irst, but later the increase suddenly rose to several hundred or several thousand units. These results seem to indicate that before excess of A is eliminated in the feces, the vitamin reserves of the y are filled and that the organism has a regulatory mechanism that vents an excessive increase in the vitamin content. In the patients considerable disturbances in the resorption such as icterus and toneal carcinosis, the amount of vitamin A excreted from the beginning was high. The concentration of A eliminated corre- nded to the degree of impairment in the resorption.

Storage.—Various experiments (Steenbock et al., '23; Wilson, '27) nitely show that the rat is able to store vitamin A in large amounts s tissues. The storage capacity for the vitamin is relatively greater he age of most rapid growth (Sherman and Cammack, '26). The appears to be the major reservoir of vitamin A storage, varying s content with the diet fed. Substantial amounts of A are also ined by the lungs and kidneys. The remarkable powers of vitamin torage are illustrated by the observation that a quantity sufficient rotect a rat for several months may be given in a single dose. Ac- ing to the findings of Chevallier and Choron ('36) the feeding itamin A results in more regular increments of the bodily store he vitamin in guinea pigs than does the feeding of carotene.

Baumann and coworkers ('34) showed that 95 per cent of the rat's

reserves were stored in the liver. Only traces could be demonstr in rats under 3 weeks of age but slightly larger amounts were fo in young whose mothers had been fed large amounts of the vita during pregnancy. The amounts stored in these animals were versely proportional to the state of depletion. At no time could m than 10-20 per cent of the vitamin administered be accounted Discrepancies between the incidence of deficiency symptoms and exhaustion of the reserves, suggested the existence of vitamin A i form which escapes detection by the usual methods. Fecal eliminat of the vitamin was small. Other and more recent studies on this s ject, using several different species of animals such as cattle, she and swine, show that vitamin A is absorbed more easily than carote

Liver diseases (Wilbur and Eusterman, '34) prevent the pro storage of vitamin A as well as the conversion of carotene into vitamin.

Of outstanding physiological interest are the experiments of R and coworkers ('36), who have studied the effect of administrati of vitamin A and of carotene upon the blood and liver vitamin and carotene of normal and diabetic dogs and humans over periods from 1 to 4 months. A given vitamin A or carotene intake w found to cause a greater rise of blood and liver vitamin A and carote in diabetic than in normal subjects, which is interpreted to indica that the power of conversion of carotene to vitamin A is diminish in diabetes.

Uterine and Mammary Transmission.—Butter fats from differe sources show little variation in vitamin A content notwithstandi the varied intake of the vitamin by the cows. Only a small fraction the total amount of A given to the cows appears in the milk. seems that if the cow's diet has a normal vitamin A content, furth additions to the diet have relatively little effect on the milk.

A survey of the literature (Dann, '32; Wolff, '32) shows that t vitamin A reserve of newborn young of various species is rather lo Although it increases during the suckling period, there is a defini limit to the amount of the vitamin which can be passed on to the of spring through the mother's milk, no matter how great her intak may be. The concentration of fat in the maternal diet has a perceptibl if scarcely important, effect on the transfer of vitamin A into mil

Normal Requirements.—The exact daily requirement of vitami A by humans has not been determined. Just as different foodstuf vary in the amount of the vitamin they contain, so individuals ma vary in their ability to absorb, store, convert, and destroy caroten and vitamin A. Nevertheless, a number of estimates of the dail human requirement for this factor are available, but for obviou reasons they vary a good deal.

he American Public Health Committee on Human Vitamin
s ('34-35) recommend 2800 International Units as satisfactory
otect infants from vitamin A deficiency.
aps and Treichler ('33) believe 1400 I. U. to be the minimal
requirement. This is considered to be rather low. Stiebeling
states that the daily requirement of adults is from 4200-5600
Growing children are allowed double the amount. Cameron
recommends an optimal intake of 5000 I. U. or more a day. The
nittee of Nutrition of the League of Nations ('36) suggests a
intake of 8700 I. U. for pregnant and nursing women.
ost of the values cited are estimates based on clinical observa-
Jeghers ('37, l.c.) recommends the use of normal dark adapta-
s an index of normal vitamin A metabolism and hence a means
imating the minimal daily requirement of healthy adults. Ac-
ng to this method 4000 I. U. represent minimal requirement
healthy adult. He advises a 50 per cent increase in the daily
for optimal results.

quirements in Disease.—The same general principles apply in
logical states as in normal individuals, but there are several
r conditions which are important, such as fever, rapid growth
et al., '33, l.c.), general infection (Wilbur and Eusterman, '34,
elevated basal metabolic rate (Wendt, '36, l.c.), and pregnancy
und and Clemmensen, '36, l.c.), in which general metabolism
reased and where an increased vitamin A intake is required.
f these conditions can lead to vitamin A deficiency even though
nount of vitamin A in the diet is theoretically adequate. In
diseases special diets are used which may be seriously deficient
Loss of appetite, gastrointestinal intolerance, and impaired
tion of vitamin A may further limit the intake of vitamin A-
ning foods. Such cases must be given careful consideration re-
g the amount of vitamin to be given, as well as the mode of
istration.

ect of Massive Intake.—A number of investigators have re-
that administration of large doses of carotene or vitamin A
es no toxic effects. Davies and Moore ('34) found that as
as 1,333,333 I. U. daily per rat showed no injurious effect.
s (Landy, '34) revealed no changes upon consumption of
I. U. daily. In a study of a group of children ranging from
ars of age, and of both sexes, dosage up to 16,000 I. U. of
amin daily had no apparent deleterious effect on their health
th, '32). These data indicate that vitamin A in itself is not
n doses much above those necessary to prevent avitaminosis A.

rapeutic Uses of Vitamin A.—Spence ('31, l.c.), Aykroyd
Frandsen ('35, l.c.), Gamboa ('30), Jeans and Zentmire ('36),

Park ('35, l.c., '36, l.c.), Jeghers ('37, l.c.), and others, have rep complete disappearance of the deficiency symptoms after treat with vitamin A-containing foodstuffs, concentrates of the variou liver oils, or carotene.

Thus treatment of this avitaminosis, in the absence of inte and hepatic diseases which prevent adequate absorption and m olism of vitamin A, consists in furnishing the vitamin in ade amounts in the diet.

If oral administration of the vitamin is impossible, it may b ministered parenterally or subcutaneously with equal efficacy.

REFERENCES

Goldschmidt (1915). Arch. f. Ophth., *90,* 345.
McCollum and Simmonds (1917). J. Biol. Chem., *32,* 181.
Mori (1922). J. Am. Med. Assoc., *79,* 197.
Daniels, Armstrong and Hutton (1923). J. Am. Med. Assoc., *81,*
Frandsen (1935). Acta. ophth. Suppl. *4,* 1.
Sweet and K'Ang (1935). Am. J. Dis. Child., *1,* 699.
Spence (1931). Arch. Dis. Child., *6,* 17.
Nicholls (1933). Indian Med. Gaz., *68,* 1933.
Nicholls (1934). Indian Med. Gaz., *69,* 241.
Frazier and Hu (1936). Arch. Derm. & Syph., *33,* 825.
Bloch (1931). Am. J. Dis. Child., *42,* 263.
Pillat (1929). Nat. M. J. China, *15,* 614; Arch. Ophth, *2,* 256, 399.
Frazier and Hu (1931). Arch. Internal Med., *48,* 507.
MacKay (1934). Arch. Dis. Child., *9,* 65.
Loewenthal (1933). Arch. Derm. & Syph., *28,* 700.
Goodwin (1934). Brit. Med. J., *2,* 113.
Sheer and Keil (1934). Arch. Dermat. & Syph., *30,* 177.
Youmans (1937). J. Am. Med. Assoc., *108,* 15.
Blegvad (1924). Brit. Med. J., *1,* 122.
DeGouvea (1883). Arch. f. Ophth., *29,* 167.
Mori (1904). Jahrb. f. Kinderh., *59,* 175.
Bloch (1917). J. Am. Med. Assoc., *68,* 1516.
McCollum (1922). J. Biol. Chem., *50,* 5.
Fridericia and Holm (1925). Am. J. Physiol., *73,* 63.
Hess (1907). Arch. f. Augenheilk., *57,* 298, 317; *59,* 143.
Holm (1925). Am. J. Physiol., *73,* 79.
Lythgoe (1937). Nature, *140,* 1045.
Jeans and Zentmire (1934). J. Am. Med. Assoc., *102,* 892.
Jeans, Blanchard and Zentmire (1937). J. Am. Med. Assoc., *108,* 451.
Edmund and Clemmesen (1936-37). "On Deficiency of Vitamin A a Visual Dysadaptation." I and II. Oxford Univ. Press, London.
Park (1935). J. Oklahoma State Med. Assoc., *28,* 359.
Park (1936a). Am. J. Digest. Dis. & Nutr., *3,* 193.
Jeghers (1937). Ann. Internal Med., *10,* 1304.

LING (1936). J. Pediat., *9*, 655.
IER and BLUMBERG (1937). U. S. Pub. Health Reports, U. S. Treasury ept., *52*, 1403.
HT (1937). Physiol. Rev., *17*, 239.
ERICHSEN and EDMUND (1936-37). Nutr. Abst. & Rev., *6*, 1079.
BACH and HOWE (1925). J. Exper. Med., *42*, 753.
BACH and HOWE (1928). Arch. Path., *5*, 239.
BACH and HOWE (1933). Am. J. Path., *9*, 275.
BACH and HOWE (1933). J. Exper. Med., *57*, 511.
OBLATT and BENISCHEK (1927). J. Exper. Med., *46*, 699.
BACH (1937). Science, *86*, 569; J. Am. Med. Assoc., *108*, 7.
ELS, ARMSTRONG and HUTTON (1923). J. Am. Med. Assoc., *81*, 828.
VILLE (1937). Science, *85*, 44.
and MCCOLLUM (1914). J. Biol. Chem., *19*, 373.
, MILLER and MCCOLLUM (1916). J. Biol. Chem., *25*, 239.
NBOCK, NELSON and HART (1922). Am. J. Physiol., *58*, 14.
HES, AUBEL and LEINHARDT (1928). Kansas State Coll. Tech. Bull., 23.
HES, AUBEL and LEINHARDT (1929). J. Nutr., *2*, 183.
LANBY (1926). J. Physiol., *61*, 24.
LE (1934). J. Nutr., *7*, 445.
IERMAN and COWGILL (1936). J. Nutr., *11*, 411.
BERT, MILLER and HUGHES (1937). J. Nutr., *13*, 1.
and GUILBERT (1937). J. Am. Vet. Med. Assoc., *91*, 193.
LANBY (1929-1930). Med. Res. Coun. Ann. Rep.
LANBY (1931). J. Am. Med. Assoc., *96*, 325; Brit. Med. J., i, 89; Brain, 247.
LANBY and KING (1934). Brit. Dental J., *56*, 538.
LANBY (1934). "Nutrition and Disease." Boyd and Oliver, London.
IERMAN and BURACK (1934). J. Exper. Med., *59*, 21.
LANBY (1930). Brit. Med. J., i, 677.
ER, STEENBOCK and PARSONS (1933). J. Nutr., *6*, 427.
R (1921). J. Path. & Bact., *24*, 306.
IS (1926). Arch. Internal Med., *38*, 785.
LANBY (1928). Physiol. Rev., *8*, 545.
LUND, KEEFER and YANG (1929). Proc. Soc. Exper. Biol. & Med., *26*, 3.
ER and YANG (1929). Nat. Med. J. China, *15*, 419.
T and YANG (1930). Arch. Ophth., *4*, 309.
(1931). Arch. Ophth., *5*, 374.
SLER, MAURER and LOUGHLIN (1926). J. Am. Med. Assoc., *87*, 476.
, KIK and WALKER (1931). Proc. Soc. Exper. Biol. & Med., *28*, 495.
LAY and MCLEAN (1925). Biochem. J., *19*, 63.
IER, DREW and MOTTRAM (1922). Proc. Roy. Soc., B, *93*, 449.
IER, DREW and MOTTRAM (1923). Brit. J. Exper. Path., *4*, 37.
ON and ZILVA (1923). Brit. J. Exper. Path., *4*, 5, 305.
ER and DREW (1923). Brit. J. Exper. Path., *4*, 271.
ONER and PEACHEY (1926). Am. J. Physiol., *76*, 145.
LAY and MACKENZIE (1922). J. Path. & Bact., *25*, Proc., 402.

Sure and Smith (1931). Proc. Soc. Exper. Biol. & Med., *28*, 439.
Morgan and Osburn (1925). J. Biol. Chem., *66*, 573.
Emmett and Peacock (1925). J. Biol. Chem., *63*, xxiii.
Kimura (1928). Acta. Schol. Med. Kioto Imp. Univ., *11*, 310.
Green (1934). Biochem. J., *28*, 16.
Osborne and Mendel (1917). J. Am. Med. Assoc., *69*, 32.
v. Leersum (1928). J. Biol. Chem., *16*, 137.
McCarrison (1931). Brit. Med. J., i, 1009.
Higgins (1933). J. Urol., *29*, 1009.
Higgins (1935). J. Am. Med. Assoc., *104*, 1296.
Oppenheimer and Polack (1937). J. Am. Med. Assoc., *108*, 349.
Smith, Parsons and Harvey (1937). J. Nutr., *14*, 373.
O'Conor (1935). J. Am. Med. Assoc., *104*, 1298.
Hou (1935). Chinese J. Physiol., *9*, 299.
Lelesz and Przezdziecka (1935). Nutr. Abst. & Rev., *5*, 610.
Long and Evans (1920). "The Estrus Cycle in the Rat." Memoirs of t
Univ. of Calif., Univ. of Cal. Press.
Evans and Bishop (1922). Anat. Rec., *23*, 17.
Macy, Outhouse, Long and Graham (1927). J. Biol. Chem., *73*, 153.
Evans (1928). J. Biol. Chem., *77*, 651.
Parks and Drummond (1926). Brit. J. Exper. Biol., *3*, 251.
Coward (1929). J. Physiol., *67*, 26.
Mason (1935). Am. J. Anat., *57*, 303.
Aberle (1933). J. Nutr., *6*, 1.
Sherwood, Brend and Roper (1936). J. Nutr., *11*, 593.
Mason and Ellison (1935). J. Nutr., *9*, 735.
Simpson and Mason (1936). Am. J. Obstet. & Gynec., *32*, 125.
Mason (1933). Am. J. Anat., *5*, 153.
Moore and Mark (1936). J. Exper. Med., *64*, 1.
Gardner and Gardner (1934). Am. J. Dis. Child., *47*, 1261.
Cameron (1934). West Virginia Univ. Bull., 34A, Ser. No. 15, p. 25.
Holmes, Piggott, Sawyer and Comstock (1936). Indus. Med., *5*, 35
Verder (1929). J. Prev. Med., *3*, 489.
Robertson (1934). Medicine, *13*, 123.
Boynton and Bradford (1931). J. Nutr., *4*, 323.
Finkelstein (1932). Proc. Soc. Exper. Biol. & Med., *29*, 969.
McClung and Winters (1932). J. Infect. Dis., *51*, 469.
Mellanby and Green (1929). Brit. Med. J., i, 984.
Green (1932). Lancet, *ii*, 723.
Findlay (1925). Brit. J. Exper. Path., *6*, 16; Proc. Roy. Soc. Med., *18*, 1
Smith and Wason (1923). J. Immunology, *8*, 195.
v. Euler (1931). Nature, *128*, 39.
Greene (1933). Am. J. Hyg., *17*, 60.
Abelin (1933). Ztschr. f. physiol. Chem., *217*, 109.
Wendt (1935). München. med. Wchnschr., *82*, 1679.
Wendt (1936). Med. Klin., *1*, 27.
Abelin (1936). Schweiz. med. Wchnschr., *66*, 1106.
Sure and Buchanan (1937). J. Nutr., *13*, 521.

R and UNGER (1937). Arch. klin. Chirurg., *189*, 405.
VALLIER and ESCARRAS (1937). Comp. rend. Soc. de biol., *125*, 1073.
MMOND, BELL and PALMER (1935). Brit. Med. J., i, 1208.
S, LEWIS and BARENBERG (1933). J. Am. Med. Assoc., *101*, 657.
AVES and SCHMIDT (1935). J. Biol. Chem., 105; Am. J. Physiol., *111*, 2, 502.
NEIDER and WEIGAND (1937). Klin. Wchnschr., *16*, 441.
NTREE (1930). J. Nutr., *3*, 265.
NDT (1937). Klin. Wchnschr., *16*, 1175.
ENBOCK, SELL and NELSON (1923). J. Biol. Chem., *56*, 327.
SON (1927). Biochem. J., *21*, 1054.
RMAN and CAMMACK (1926). J. Biol. Chem., *68*, 69.
VALLIER and CHORON (1936). Compt. rend. Soc. de biol., *121*, 1015; *123*, 9.
MANN, RIISING and STEENBOCK (1934). J. Biol. Chem., *107*, 705.
BUR and EUSTERMAN (1934). J. Am. Med. Assoc., *102*, 364.
LI, PARIENTE, BRANDALEONE and DAVIDSON (1936). J. Am. Med. Assoc., 6, 1975.
N (1932). Biochem. J., *26*, 1072.
LFF (1932). Lancet, *ii*, 617.
. Pub. Health Comm. on Human Vitamin Needs (1934-1935). Am. ub. Health Year Book.
PS and TREICHLER (1933). Texas Agr. Exper. Sta. Bull. 447.
BELING (1936). Bureau Home Econ. Rep., U. S. Dept. Agric.
IERON (1935). J. Am. Dietetic Assoc., *77*, 189.
rition Committee, League of Nations (1936).
IES and MOORE (1934). Biochem. J., *28*, 289.
DY (1934). Dept. Bact., Ohio State Univ. (unpublished data).
BEATH (1932). J. Dental Res., *12*, 723.
KROYD (1930). J. Hyg., *30*, 357.
MBOA (1930). J. Philippine Is. Med. Assoc., *10*, 235.
NS and ZENTMIRE (1936). J. Am. Med. Assoc., *106*, 996.
K (1936b). J. Oklahoma State Med. Assoc., *29*, 129.

CHAPTER

XIV

Chemical Nature of Vitamin D

IN DISCUSSING the discovery of vitamin A it has been poin out that the deficiency state involving this nutrient long anteda any definite knowledge of its etiology, and that, while some succ was realized in its dietary treatment, it was not until after the expe mental production of the pathological state in animals that rap progress was possible in the accumulation of exact knowled of the dietary factor involved. Liver was known before the Christi era to be a specific remedy for night-blindness; Lind and othe knew of the virtues of fresh vegetable foods as preventives of scurv and Takaki eradicated beriberi from the Japanese navy by institutir a dietary reform. The experimental production of these diseases animals was promptly followed, in every instance, by extremely rap advance of knowledge, both of the nature of the missing substance diet, and of the physiology and pathology of these states. The histo of the advancement of knowledge of rickets and of antiricketic su stances followed a comparable course.

The use of cod liver oil as a therapeutic agent appears to da from its employment by Darby in 1789, at the Manchester Infirmar It was then a "medicine" whose usefulness was not well defined. I use for rickets was first reported in 1824 by Schuette and althoug it continued to be administered for this purpose, its specific valu remained unrecognized for nearly a century. Thus, Howland ('20 held reservations when he said, "Cod liver oil has been used empirical for a long time. It is regarded by many physicians as a specific i the treatment of this disease (rickets). This is perhaps too strong praise, but there is no doubt as to its usefulness. Its effect, howeve is not very prompt or marked." Yet in 1918 Mellanby had produce rickets in puppies, and had compared the value of several kinds o

for its prevention and cure, and had proven clearly the marked riority of cod liver oil for these purposes. Since the merits of ils tested correlated fairly well with their content of vitamin A, it natural for him to infer that rickets resulted from a deficiency is substance. Korenchevsky ('22) and Goldblatt ('23) confirmed results of Mellanby in demonstrating that deficiency of a fat- le vitamin alone would cause the disease, all other factors neces- for growth being supplied. Hess and Unger ('20) pointed out infants developed rickets while receiving a liberal intake of milk an ample supply of "fat-soluble vitamin."

n 1921 Sherman and Pappenheimer found that young rats de- ed rickets when fed a diet containing little phosphorus and a rous amount of calcium. A healing response was obtained by provision of a suitable amount of phosphorus. Shipley, Park, Collum and Simmonds ('22) showed that there were two kinds ickets,—one due to feeding a diet deficient in calcium, but con- ng a normal or excessive amount of phosphorus; the other due eeding a diet deficient in phosphorus and containing a normal xcessive content of calcium (low-calcium rickets; low-phosphorus ts). Shipley, Park, McCollum, Simmonds and Parsons ('21) d that whatever the defects of the salt composition of the diets loyed in the production of experimental rickets in young rats, effect of the addition of cod liver oil was greatly to improve growth and condition of the animals, and to shift the pathological ition of the bones toward the normal state. In a rapid series of ies involving many modifications of diets, they concluded that content and ratios of calcium and phosphorus in the diet, to- er with the presence or absence of some organic factor contained od liver oil, constituted the essential factors in the etiology of ts in rats under experimental conditions. That this organic factor not vitamin A was shown by the fact that cod liver oil which been treated with a stream of air bubbles while hot, no longer ained vitamin A, but was still potent in the prevention or cure ckets (McCollum, Simmonds, Becker and Shipley, '22). The anti- tic factor which they thus demonstrated was called vitamin D.

ormation of Vitamin D. The Role of Sunlight and Other rces of Ultraviolet Light.—Since the earliest times certain physi- s recognized the value of sunlight in the treatment of disease. ('23) has reviewed the older medical literature on this subject. n (1890) as the result of a topographic study of the distribution ickets, concluded that sunlight should be regarded as a thera- ic agent. Huldschinsky ('19) reported that ultraviolet rays ex- l a curative action on rickets. A knowledge of the fairly numerous rts on the curative effect of sunlight and of ultraviolet rays on

rickets led Shipley, Park, Powers, McCollum and Simmonds to test light treatment on the development of rickets in rats on diets which quickly induced severe and acute rickets in ani kept indoors. They confirmed the earlier findings by the observa that rats on these diets did not show rickets when exposed to sum sunshine in Baltimore. The experimental production of rickets b now on a sound basis, the observation on the effect of light was confirmed by others. The manner in which ultraviolet light exe its effect, however, still remained a mystery. Simultaneously independently, Hess ('24) and Steenbock ('24) had the happy thou to test the effect of irradiating a rickets-producing diet with ultravi rays and made the remarkable discovery that this resulted in ferring on such diets antiricketic properties.

Soon after reporting their fundamental observations both of th investigators studied the effect of irradiating separately protein, car hydrates, fats, and mineral salt mixtures, each of these materials be included, singly, in the diet of ricketic rats. From these stud emerged the fact that it was the fat fraction which became "activate in the sense of possessing antiricketic properties. The next logical s was immediately taken, to determine what constituent of fats, v fatty acids, glycerol, or cholesterol was acted upon by ultraviolet ra to produce this effect. It was found that the unsaponifiable fracti of fats only was converted by light into an antiricketic substan Hess ('24, l.c.) found that inert oils, e. g., cottonseed and linseed, a quired curative properties on irradiation. Hess, Pappenheimer an Weinstock ('22) had extended the observation of Huldschinsky, bas upon radiographs, that sunlight and the light from a quartz mercu arc could cure rickets. They demonstrated that the effective wav lengths of light are in the shorter ultraviolet region of the sol spectrum and that the activation of foodstuffs depended upon th same short wavelengths which were effective in the direct cure rickets.

Further study showed that cholesterol and phytosterol becam antiricketic on irradiation (Hess, Weinstock and Helman, '25). Stee bock and Black ('25) found that cholesterol, after esterification, cryst lization, and regeneration from its ester, could still be activated. F a short time it seemed that cholesterol and phytosterol were pr vitamin D. Bills ('35, '38) has written excellent reviews of the r searches which led to the identification of the chemical nature vitamin D. No attempt will be made, for lack of space, to mentio here all of the contributions to this field of knowledge. The mo significant discoveries only will be cited.

Schultz and Morse ('25) showed that the absorption spectrum

inary cholesterol is banded, and that there are two maxima of orption at approximately 294 to 283 mμ. They noticed that two itional faint inflections on their densitometer tracings, after brief diation, gave way to general absorption. This they explained by alternative postulates that either the cholesterol had become at t half metamorphoses, or else ". . . the substance in which the orption spectrum is changed may be a small amount of impurity he cholesterol which is not removed by repeated crystallization n alcohol, and which is exceedingly absorptive."

Bills ('35, l.c.) pointed out the coincidence that on December 10, , three researches from different laboratories confirmed the hy- nesis that provitamin D is an "impurity" in ordinary cholesterol. enheim and Webster ('26) reported that cholesterol which had n regenerated from its dibromide no longer showed the charac- tic absorption spectrum and could not be made antiricketic by diation. They also showed that ergosterol, a sterol found abun- tly in fungi such as ergot oil and yeast, could be activated. They not determine its extraordinary potency as an antiricketic agent did not recognize it as the "impurity" in ordinary cholesterol.

Heilbron, Kamm and Morton ('26) reported that when cholesterol epeatedly recrystallized the substance which is responsible for characteristic absorption spectrum tends to accumulate in the soluble fraction. They also observed a third absorption maxi- n at 269 mμ. Irradiation destroyed the three bands, leaving only eral absorption.

Pohl ('26) detected three absorption bands in cholesterol, and d that they faded as irradiation was continued. Since the absorp- bands disappeared when only a trivial amount of cholesterol d have been transformed, he concluded that the absorbing sub- ce was present only in minute amount. He also found that puri- ion of cholesterol through its dibromide removed the absorbing tance. These three studies established the fact that the provitamin f cholesterol and phytosterol is a substance associated with these ls but in small amount. Pohl ('27) pointed out that the three rption bands of cholesterol of ordinary purity were also exhibited rgosterol, which showed them in vastly greater intensity. Almost ltaneously Windaus and Hess ('27) and Rosenheim and Webster) announced observations that irradiated ergosterol is of extraordi- potency as an antiricketic agent, and the conclusion that the urity" in ordinary cholesterol, to which it owed its characteristic rption spectrum, is ergosterol and that this is the precursor of min D.

Bills, Honeywell and MacNair ('28) confirmed these findings and,

employing a more refined technic than any hitherto used, detec a fourth absorption maximum at 260 mμ. They compared the p tions of the four bands of ordinary cholesterol with those of p ergosterol, and found them identical, making the series 293.5, 2 270, and 260 mμ. They further observed that ordinary choleste and ergosterol-free cholesterol plus added ergosterol, when dissol in acetone and boiled with potassium permanganate, showed fad of the absorption bands at essentially the same rate. On the basis these findings the belief was established that ergosterol, which clearly a form of provitamin D, is the "impurity" in ordinary cho terol to which it owes its antiricketic properties on irradiation.

Notwithstanding the apparent proof that ergosterol is a consta contaminant of ordinary cholesterol, the assumption proved erroneo In 1930 Muessehl and Ackerson reported that amounts of irradiat yeast and irradiated ergosterol which, calculated on the basis of b assays on ricketic rats, were equivalent to 10 to 50 per cent of c liver oil in the diet, failed to prevent leg weakness in chicks. Masse gale and Nussmeier ('30) found that it was necessary to administ the equivalent of 200 per cent cod liver oil in the form of irradiat ergosterol to produce in chicks the effect secured by 2 per cent c liver oil. Several other workers secured similar results. Bethke, Reco and Kennard ('33) made a critical study of the comparative an ricketic values of these two sources of vitamin D, and found that required 15 to 20 times as many rat units of vitamin D in the for of irradiated ergosterol as of cod liver oil to insure normal bone fo mation in chicks.

Employing this new method of comparative assays with rats an chicks, Waddell ('34) tested irradiated cholesterol as a rickets-pr ventive for chicks, and demonstrated that it is much more effecti than an equivalent number of rat units of irradiated ergoster Irradiated cholesterol, he found, is at least as effective as the vitami D of cod liver oil, hence the provitamin, which had been presume to be ergosterol, is actually another substance. 7-dehydrocholester first synthesized by Windaus, Lettré and Schenck ('35), appears to b the principal activatable sterol or provitamin D in cholesterol. Th is the form present in the skin and is probably the chief form foun in fish oils. Irradiated cholesterol and activated 7-dehydrocholester exhibit the same spectral absorption bands as ergosterol. This produ of irradiation proved to be a highly potent antiricketic substanc These findings suggest that 7-dehydrocholesterol is the provitamin in ordinary cholesterol.

Products of Irradiation of Ergosterol.—The elucidation of th chemical changes which ergosterol undergoes upon treatment wit

aviolet light was brought about by an English group (Askew l., '30, '32; Angus et al., '31) and by a German group of chemists indaus et al., '31). These changes occur in the following order:

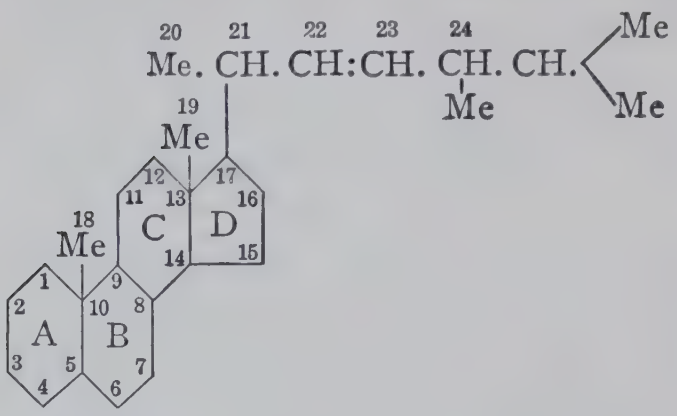
osterol → Lumisterol → Tachysterol →
Calciferol → Toxisterol → Suprasterols
(vitamin D_2) (Substance 248) (I and II)

umisterol resembles ergosterol in its absorption spectrum, but only two maxima instead of four. It appears to be devoid of anti-etic activity, but it is converted by irradiation into vitamin D_2. orms with calciferol (D_2) a definite addition compound. Tachys-l shows an absorption band at 280 mμ. It appears to have no anti-etic action, but may have a slight toxic effect. Toxisterol, Substance is a product which shows absorption of great intensity at 248 the band which coincides with the destruction of antiricketic ncy. This compound is related to the isoergosterols but has one t of difference, namely, that it does not precipitate with digitonin. s most readily formed when ergosterol in alcohol is irradiated. rasterols I and II represent the products of over-irradiation. These tances are not antiricketic and are slightly toxic. Each of the rmediate irradiation products in the above series has been isolated studied, as have also the suprasterols.

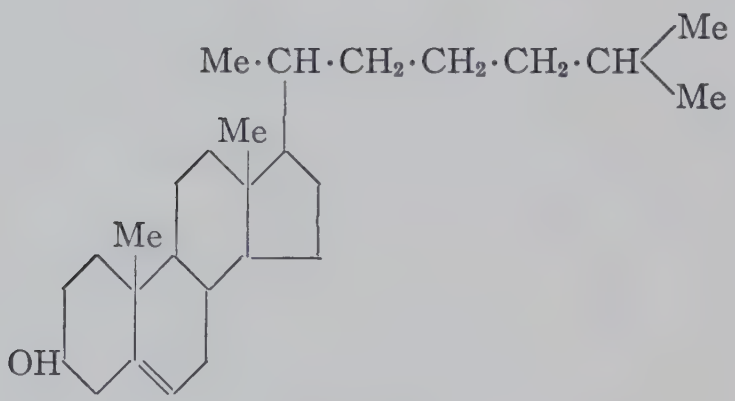
Vitamin D may be formed by other means than by irradiation n ultraviolet light. When cholesterol is exposed to cathode rays ecomes antiricketic (Knudson, '27; Knudson and Coolidge, '27; udson and Moore, '29; Hoffman and Daniels, '36, McQuarrie, mpson, Stoesser and Rigler, '37). The activating effect of cathode upon ergosterol has been shown not to be the result of exposure to aviolet radiation but is due chiefly to the rays themselves. The min D potencies attainable by the action of cathode rays are lower n those attained by ultraviolet light irradiation. Maisin and co-kers ('30) have reported that ergosterol may be converted into min D by radium emanation.

Extraordinary advances have been made in the study of the struc-l relationships of the sterols, to which class of compounds vitamin n its several forms belongs. The formulae for the more important pounds related chemically to vitamin D are presented to show r relationships:

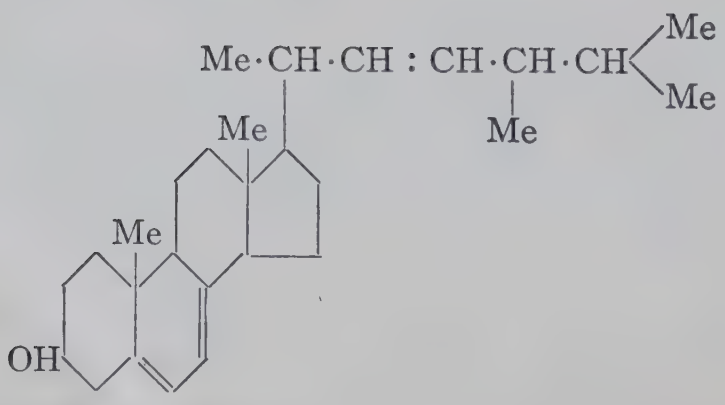
Vitamin D Formulae



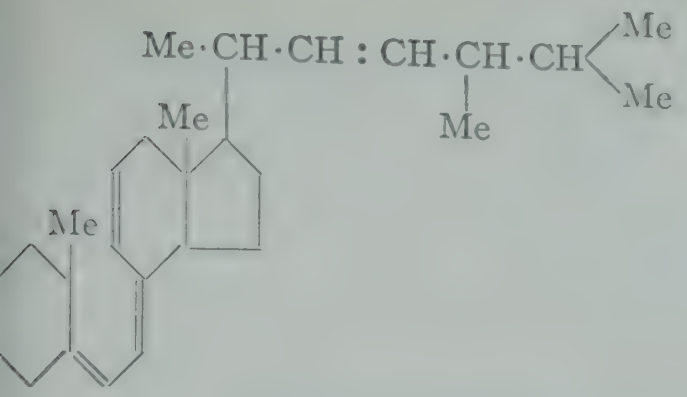
The sterol ring structure. Side chain shown as in ergosterol.



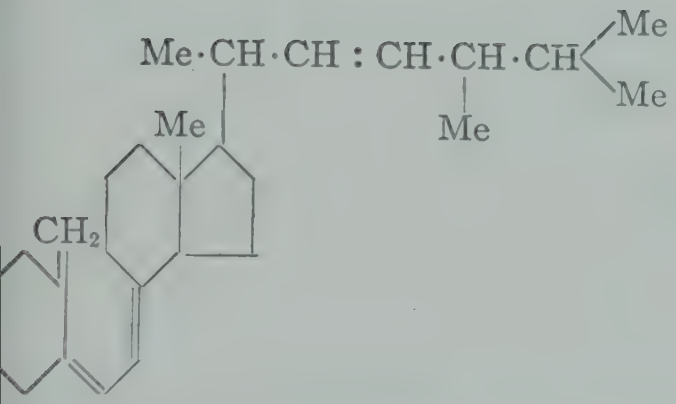
Cholesterol.



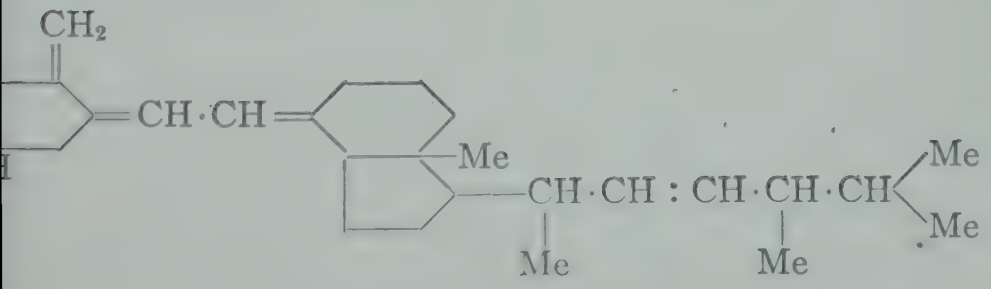
Ergosterol, lumisterol, pyrocalciferol and isopyrocalciferol.



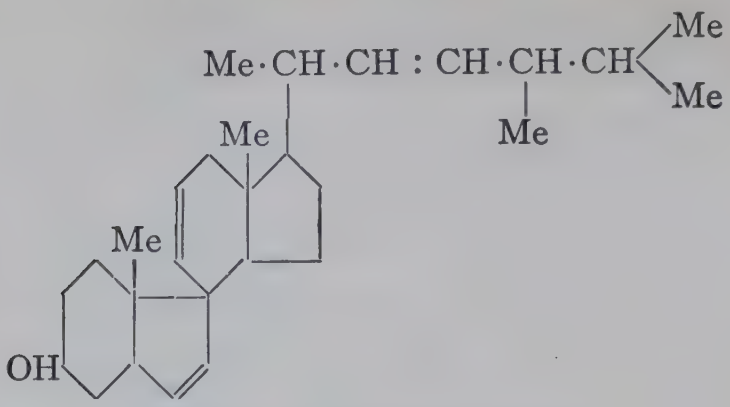
Tachysterol (a tentative formula).



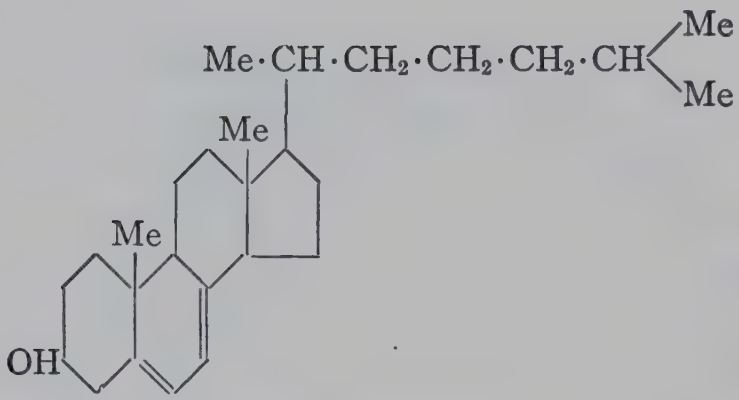
Calciferol (vitamin D_2). Usual, or steroid, formula.



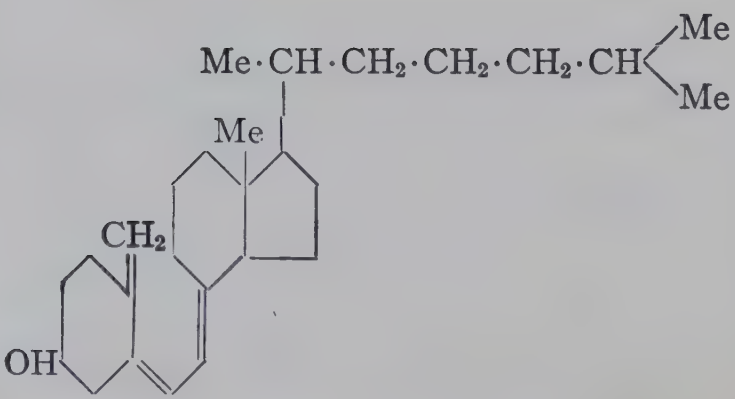
Calciferol, stilbenoid formula.



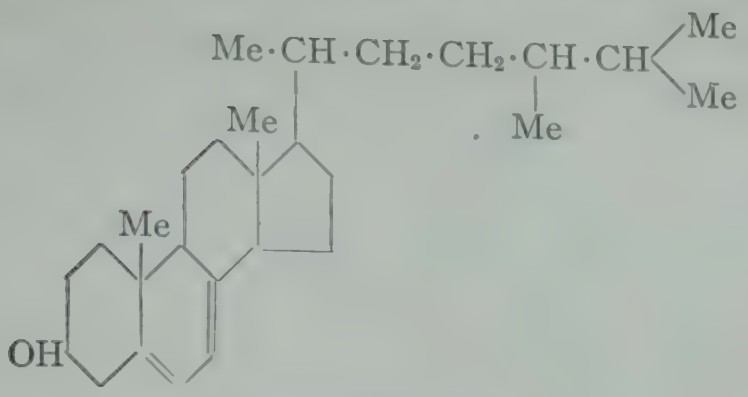
Suprasterol I (a tentative formula).



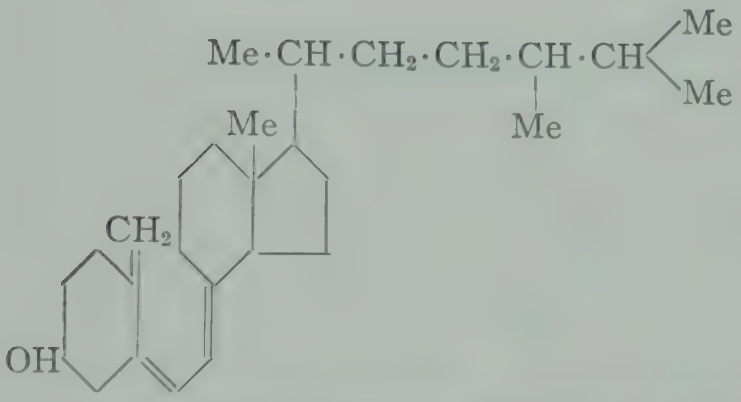
7-Dehydrocholesterol.



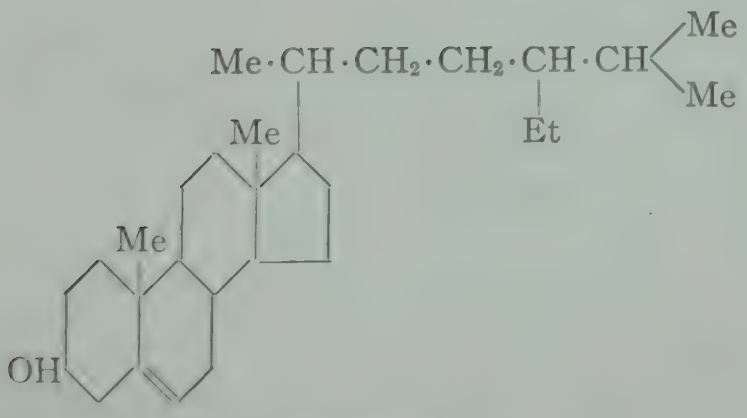
Activated 7-dehydrocholesterol (vitamin D_3).



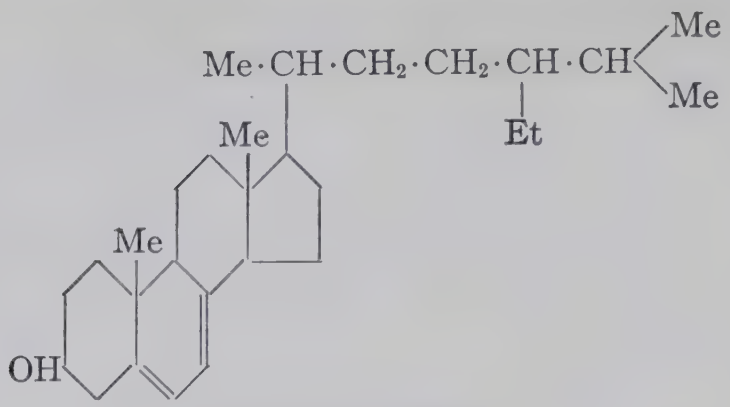
22-Dihydroergosterol.



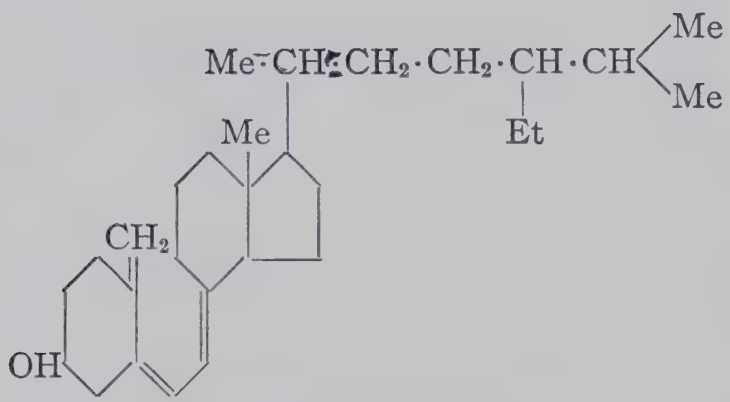
Activated 22-dihydroergosterol (22-dihydrocalciferol or vitamin D_4).



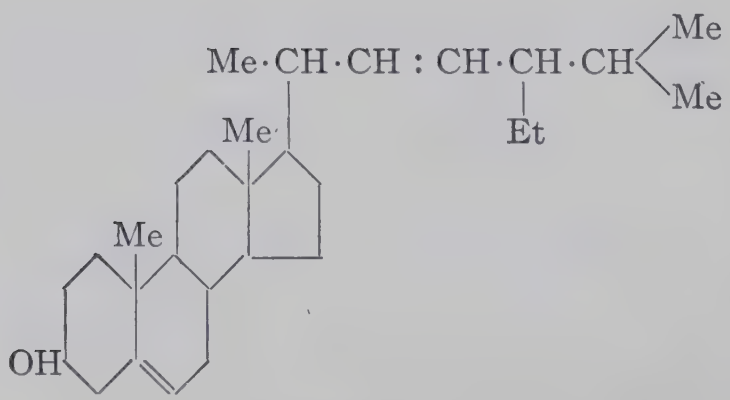
Sitosterol.



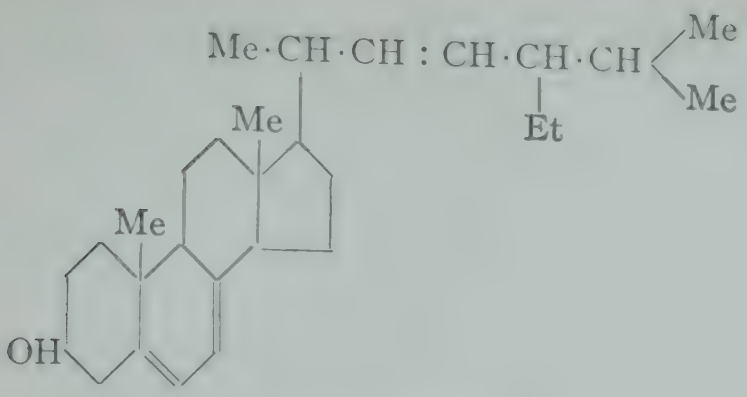
7-Dehydrositosterol.



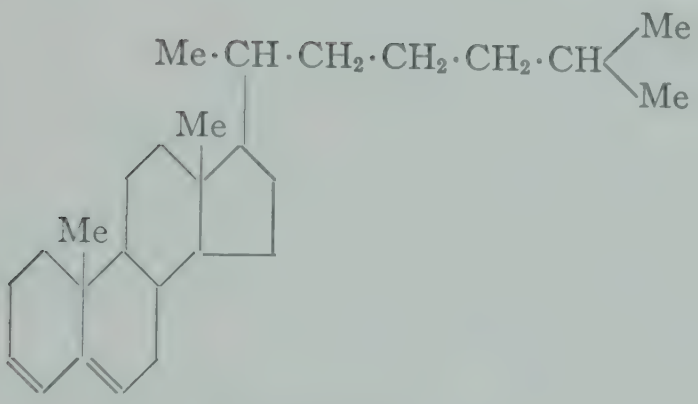
Activated 7-dehydrositosterol.



Stigmasterol.



7-Dehydrostigmasterol.



Cholesterilene.

he Various Forms of Antiricketic Substances.—McCollum and ssociates designated the substance in cod liver oil which pos- antiricketic properties "vitamin D." As has already been men- l, further researches have shown that several sterols possess this rty. During the period of attempting to separate the compounds ng from irradiation of ergosterol, the term vitamin D_1 was to the first antiricketic substance isolated from irradiated ergo- which consisted of a molecular compound of vitamin D with ond irradiation product called lumisterol. The molecular com- l later proved to be calciferol, now also known as vitamin D_2. ated 7-dehydrocholesterol is vitamin D_3, and vitamin D_4 is acti- 22-dihydroergosterol (22-dihydrocalciferol).

lciferol is the form of vitamin D which is sold in the pharma- al trade as viosterol in oil. Although crystalline esters of the of vitamin D present in tuna and halibut liver oils have been ob-

tained, no crystalline preparations of the free vitamin from t sources have as yet been described. The evidence is very convin however, that the form of the vitamin in these oils is activated 7-d drocholesterol. This form is the most important naturally occu one. It has been isolated by Windaus and Bock ('37a) from the s mixture of hog skin, and by Boer and his coworkers ('36) f cholesterol of unstated origin. It appears, therefore, that the vitamin in skin, feathers, wool, etc., is 7-dehydrocholesterol, and to the activation of this sterol that the antiricketic effect of ultrav rays of sunlight is due. Hence, various texts and other publicat are in error when they state that the activatable substance in ski ergosterol. It has been shown that vitamin D_3 is the form foun irradiated cholesterol obtained from butter, brain, fish oils, etc. the form present in irradiated foods of animal origin, including m 22-dihydrocalciferol, vitamin D_4, is the form found in irradia vegetable foods, except in yeast or other fungi, which may be pre in certain foods, and which, because they contain ergosterol, wo contribute vitamin D_2 on irradiation.

Sitosterol is the name of the ordinary sterol found in plants (Gr *sito-,* grain). It has been shown to consist of at least three isom Phytosterol was long used as a general name for plant sterols, sitosterol differentiates those sterols occurring in plants, other th fungi, from the phytosterol of yeast which is composed of ergoste and zymosterol. Stigmasterol occurs in soy bean oil, the oil of Calabar bean and probably elsewhere. It is of interest to note th ergosterol was isolated from the oil of ergot by the French chem Tanret in 1879, but although he described its properties he did know what an extraordinary substance he had discovered. Of inter also is the report by Windaus and Bock ('37b) that the provitamin of the higher plants appears to be ergosterol and not 7-dehydrosi sterol as had been anticipated.

Physico-Chemical Properties of the Antiricketic Substances. *Natural Vitamin D:* The physico-chemical properties of the va ous forms of natural vitamin D found in the liver oils of many spec of fish appear to be identical. The vitamin is soluble in alcohol, a tone, and ether; however, the degree of solubility depends somewh upon the source from which the vitamin is extracted. This fact resists oxidation more strongly than vitamin A, as has been sho by the failure of aeration or treatment with hydrogen peroxide effect appreciable loss of antiricketic activity. Mild hydrogenation the action of reducing agents such as hydrogen sulfide, sulfur dioxi or formaldehyde, do not readily destroy it. Nitrous fumes, on t other hand, produce appreciable loss. The vitamin is thermostab in neutral medium, being able to withstand the direct steaming proc

in rendering fish livers. In acid medium gradual destruction place, the rate varying with the reaction and temperature. It affected by strong alkali even at high temperatures. Saponifica- is used to separate the antiricketic fraction of the original oil is carried in the unsaponifiable portion. This is composed y of sterols, chief among which is cholesterol. Ergosterol, very r in composition to cholesterol but more unsaturated, is found atively large amounts in mushrooms, fungi, yeast, and especially Highly purified vitamin D concentrates are optically inactive how an absorption spectrum in the region 260-270 mμ.

tamin D Produced by Irradiation: As has been stated else-, the properties of vitamin D are exhibited by at least eleven ent sterol derivatives. The properties of the two considered to most practical significance,—irradiated ergosterol and irradiated ydrocholesterol, will be described here. Before discussing these, ief physical and chemical properties of untreated ergosterol and terol respectively, will be briefly mentioned.

tamin D_2: Ergosterol, the provitamin of calciferol (D_2), is a , crystalline solid melting at 166°-183° C. according to the degree dration. It is sparingly soluble in alcohol and other organic sol- It is precipitated by digitonin. It is levorotatory and exhibits a adsorption in the ultraviolet with well-defined maxima at 260, 82, and 293.5 mμ. Many isomers of ergosterol exist, some of occur naturally and some of which may be prepared in the lab- y. Of the latter group some are activatable and some are not. terol itself has no antiricketic activity. After it is irradiated with iolet rays, some of its physical and chemical properties are ed. The crude irradiation product after removal of unchanged erol is a nearly colorless resin. It melts at 166° C. and has a r solubility in organic solvents than the original ergosterol. It no forms an insoluble compound with digitonin. The optical rota- s changed to weakly levo- or even dextrorotatory. The different of vitamin D produced by irradiation vary somewhat in anti- ic potency. This may be the result of incomplete irradiation or it e due to the presence of impurities. The antiricketic potency of ted ergosterol seems to be just as stable as that of the fish liver Even the dry irradiation products withstand storage either *in* or in air for months without appreciable destruction of the in. Temperatures of 180° C. or higher, or a combination of n with high temperature will, however, destroy it. When ergo- is irradiated a number of substances are obtained, the compounds d depending on the wavelengths of light used. Calciferol (D_2) form of vitamin D yielded by irradiated ergosterol. Calciferol rystalline substance. In appearance it is indistinguishable from

ergosterol. After several months of exposure it changes to a b resin. It is dextrorotatory and is isomeric with ergosterol, havin formula $C_{28}H_{43}OH$. Like most other products of irradiation, it not precipitate with digitonin. It shows a strong ultraviolet absor with a maximum of 265 mμ. Its antiricketic potency is 40,000 I.U mg. Like irradiated ergosterol it is inferior, rat unit for rat un cod liver oil for the chicken. Its absolute potency for the rat is mous, being about 400,000 times that of average cod liver oil.

Vitamin D_3: This form of vitamin D is distinctly different f that produced by the irradiation of ergosterol. It is formed upon activation of cholesterol or 7-dehydroxycholesterol. Cholesterol itse a mono-hydroxy-secondary alcohol with a terminal vinyl group a double bond and containing three saturated hexa-carbon ring is a white crystalline substance which melts at 148.5° C., and *in va* at 300° C. it sublimes unchanged. It is levorotatory and is sol in the common organic solvents. It cannot be saponified and is cipitated by digitonin. Its absorption spectrum consists of the s bands as ergosterol.

The compound 7-dehydroxycholesterol which has been isola from tuna fish oil, as well as synthesized, is a cholesterol derivat identical with ergosterol except in the side chain. It possesses the sa nuclear arrangement of double bonds as ergosterol. It is a C_{27} co pound lacking the side chain methyl group as is characteristic of a mal sterols which have one fewer carbon atom than the vegeta sterols (C_{28}). In its absorption spectrum, its photochemical oxidati and dehydrogenation as well as its conversion into a series of dihyd compounds, 7-dehydroxycholesterol closely resembles ergosterol. T changes effected by irradiation of 7-dehydrocholesterol proceed with ergosterol, i. e.: 7-dehydrocholesterol → lumisterol$_3$ → tachyster → anhydride vitamin D_3 (Windaus, et al., '37). Lumisterol$_3$ clos resembles lumisterol in its spectral and other properties, but gives addition product with vitamin D_3. Both irradiated cholesterol and dehydroxycholesterol acquire powerful antiricketic properties on radiation. Vitamin D_3 has practically the same absorption spectrum calciferol (D_2) and presumably an analogous structure. Of the vario forms of vitamin D it has the highest antiricketic activity. Its relati species effectiveness is similar to that of the natural vitamin D fou in cod liver oil.

Other Forms of Vitamin D: The various other forms of vitam D although of theoretical significance to the chemist appear to be no practical importance because of their low antiricketic activity. Th are:

1. (D_4). Activated 22-dihydrocalciferol. (Possibly occurring plant sterols and hence in irradiated vegetable foods.)

7-dehydrositosterol. It is but slightly antiricketic for rats.
Cholesterilene. This was first prepared by Bills ('26) by treat-
of cholesterol with fuller's earth. Its antiricketic activity is slight.
('36) has shown that cholesterilene sulfonic acid and its salts
mewhat antiricketic for rats, but more effective for chicks. It
ot require irradiation.
7-hydroxycholesterol (or an impurity associated with it).
d by McDonald (cited by Bills, '37).
Cholesterol, freed from its normal provitamin, but not heated to
ce a new one, gives, upon irradiation, a vitamin D which is less
ve for chicks, rat unit for rat unit, than the vitamin D which is
d by irradiating either unpurified cholesterol or heat-treated
terol (Stavely and Bergman, '37).
A product prepared by the treatment of ergosterol with nitrites.
low potency, and is believed to be different from the ordinary
of vitamin D. which are destroyed by nitrites (Bills, '35, l.c.).
The irradiated product formed by heat treatment of 7-ketocho-
l acetate, and isobutyl-magnesium bromide (Weinhause and
sch, '36).
Windaus and Trautman ('37) mention, among other provita-
22-23-oxidoergosterol. It is but feebly antiricketic.
Bills, Massengale, Hickman and Gray ('38) reported the isola-
f a new vitamin D in cod liver oil which may differ from the
cally known forms in the absence of the usual side chain.
e Plant Origin of Vitamin D.—The presence of vitamin D in
ver oils has been difficult to explain. Steenbock and Black ('25,
ggested that the vitamin might originate from the solar irradia-
f microscopic plants and animals living near the surface of the
nich are known as plankton.
me evidence (Belloc, et. al., '30; Copping, '34) is available indi-
the presence of vitamin D in plankton of animal form. Assays
nkton of plant form (Leigh-Clare, '27; Drummond and Gun-
30) however, have been consistently negative.
interesting observation has been reported by Darby and Clark
They found vitamin D in *Sargassum* weed, a large marine
growing in the Caribbean Sea. The probability that this plant
contain the vitamin was suggested by the observation that there
siderable penetration of solar ultraviolet in the very clear sea
at the Torbugas Islands. The *Sargassum* weed collected off the
gas Islands yielded oils having definite antiricketic activity for
. Since masses of this weed harbor numerous small marine ani-
f various forms, it is suggested that the vitamin is progressively
rred from the plant to the small animals, thence to the larger

Vitamin D has not been found in living tissues of land p
However, since such plants are known to acquire some vitam
after cutting and exposure to the sun, a possible explanation o
presence of this factor in *Sargassum* is that vitamin D is forme
solar irradiation only in dead portions of the weed.

The Origin of Provitamin D in the Body.—The mechanism i
known by which 7-dehydrocholesterol is formed in the body but
presumably formed from cholesterol. The function of cholester
metabolism is still somewhat of a mystery. Its presence in l
amounts in brain and nerves shows that it is an important struct
material in these tissues. It is present in small amounts in all tiss
and it was long ago conjectured that cholesterol is convertible into
bile acids, a view which is established by recent researches. Sch
heimer ('31, l.c.) has proved that coprosterol, which is isomeric v
dihydrocholesterol, and which was long supposed to be formed by
action of bacteria in the intestines, where it is relatively abund
actually is formed in the body and excreted into the gut. He isola
the large intestine of dogs, and after repeated rinsing, the anus v
closed. The antibacterial action of the intestinal wall was sufficient
destroy the few remaining bacteria and it became sterile. After
or two months the animals were sacrificed and the contents of
isolated colon were examined. From 60 to 70 gm. of a light yello
wax-like mass, were obtained, which represented the desiccated int
tinal secretion, that normally would have been mixed with the fe
and have been eliminated. This material contained everything that w
secreted into the intestine but which could not be reabsorbed. From
he isolated considerable amounts of dihydrocholesterol, but no tra
of coprosterol. It thus appears that coprosterol is the product of ba
terial action, not on cholesterol, but on dihydrocholesterol. Coproster
represents the cis- and dihydrocholesterol the isomeric trans- form
the same substance.

Distribution of Vitamin D in Foodstuffs.—Vitamin D is pre
ent in only a few foods which are commonly eaten, namely, in th
flesh of certain fish and in eggs. Although fish liver oils have foo
value as sources of energy as well as of vitamin D, they cannot strict
be considered foods, according to our dietary habits. The flesh of suc
oily fishes as sardines, herring, tuna, and salmon, fresh or canned, co
tain considerable, but variable amounts of the vitamin. Less general
used fishes such as menhaden, lamprey, and eel, and doubtless man
other fishes, contain it. Fish roe contains the vitamin in fair amount
as do turtle's eggs and hen's eggs (Daniel and Munsell, '37). In th
latter it has been shown that the season of the year and the system
feeding affect the vitamin D content. DeVaney, Munsell and Titu
('33) reported for February and June eggs, 140 I.U. and 390 I.U

ctively, per 100 gm. All the vitamin is in the yolk. Drake (cited ark, '38) estimated that approximately five eggs supplied the in D equivalent of one teaspoonful of cod liver oil. When erol (viosterol), fish liver oils, or irradiated foods are fed to the vitamin D is transferred to some extent to the eggs. Cod liver much less effective in this respect than calciferol. Irradiation of en by the ultraviolet rays of sunlight increases the vitamin D con- of her eggs.

rdinary milk contains but little vitamin D. Campion and co- ers ('37) reported that milk from cows kept out of doors in June, ngland, contained 17 to 26 I. U. per quart, whereas milk from kept indoors contained 5 to 8 I. U. per quart. Bechtel and Hop- ('36) found 5 I. U. per quart of winter milk, and 40 I. U. per t in summer milk. Pasture feeding has almost no influence on the ent of vitamin D in milk, but exposure of the cows to sunlight ases it. The vitamin D content of milk may be increased by feed- rradiated yeast to the cow. Average values obtained by assay of in D milks produced in the following ways are:

Yeast-fed cows	432 I. U. per quart
Cod liver oil concentrate-fed cows	405 I. U. " "
Irradiated milk	135 I. U. " "

n the milk is irradiated, or cod liver oil concentrate is fed, the of the vitamin is probably activated 7-dehydrocholesterol.

he vitamin D of milk is in the fat moiety, and hence passes into r. Average butter contains about 80 I. U. per 100 gm., or about r cent of the value of ordinary cod liver oil.

Iammalian liver contains 10 to 45 I. U. of vitamin D per 100 gm. r glandular organs of land animals contain very little of the in, but it probably occurs in significant amounts in the flesh of and animals which subsist largely upon fish. Seal flesh is high itamin D. The most abundant natural sources of vitamin D are liver oil and percomorph oil.

lant foods and vegetable oils are markedly deficient in vitamin D.

itamin D Milk.—As a food of general and regular consumption, is an appropriate carrier of vitamin D especially for infants and ing children. However, since vitamin D milks usually provide t one-third the usual therapeutic dose of D, they are chiefly pre- ive agents. Health commissioners generally approve the fortifica- of milk with vitamin D. This is a sound policy and it has been rsed by the Council on Foods of the American Medical Associa- ('37): "Of all the common foods available, milk is most suitable carrier of added vitamin D. Vitamin D is concerned with the zation of calcium and phosphorus, of which milk is an excellent

source. The Council has recently made the decision that for the pr milk is the only common food which will be considered for ac ance when fortified with vitamin D." The Council recommends 400 to 500 I. U. daily as a reasonable addition to the diet during c hood and later years.

Several processes are in commercial use for increasing the ricketic potency of whole or evaporated cow milk. They are the f ing of activated yeast to cows, a small fraction of the vitamin (calciferol) being transmitted to the milk; ultraviolet irradiation the milk itself; and fortification of the milk with irradiated ergost or fish liver oil concentrates; a fourth method involves the addi to milk of ergosterol activated by exposure to cathode rays from Coolidge tube. Direct irradiation produces milk containing 135 I. per quart as a maximum and therefore infants taking this milk n additional vitamin D. Irradiated yeast, if fed in controlled amou produces milk of satisfactory antiricketic potency. If fortified milk standardized to contain 400 I. U. per quart of whole milk it is adequate antiricketic agent. Milk of such activity will prevent rick if ingested in amounts of only one pint daily although at this le the margin of safety is probably small.

Determination of Vitamin D Potency.—There is no chemi method for the assay of vitamin D potency of any product. Her some type of animal assay, or bio-assay, must be employed. All p cedures depend upon the effect of a given dose of vitamin D on c cification of the bones of severely ricketic rats. The first diet suitab for producing rickets for the purpose of bioassay of vitamin D w Diet 3143 of McCollum and his associates. This consisted of:

	Per Cent
Whole wheat, ground	33.0
Yellow maize, ground	33.0
Gelatin	15.0
Wheat gluten	15.0
Calcium carbonate	3.0
Sodium chloride	1.0

One hundred gm. of this ration contains 1.22 gm. of calcium and 0. gm. of phosphorus. Weight ratio Ca:P, 4.04:1.

Steenbock and Black proposed a ricketogenic diet (No. 2965) whi fulfills the dietary requirements above mentioned for the productio of rickets. It consists of the following substances:

	Per Cent
Yellow maize, whole seed, ground finely	76.0
Wheat gluten	20.0
Calcium carbonate	3.0
Sodium chloride	1.0

Observations of the deposition of bone as revealed by visual examion of bone sections stained with silver nitrate, radiographs of the es of the living rat, and determination of the ash content of the es, after a suitable interval following the administration of vitamin test animals, have been the criteria on which most bio-assays have n based. Then there is the possibility of both a preventive and a tive procedure. Many methods have been advanced, which con- of modifications of one or another of these procedures. Any of ral methods may be reliable in the hands of an experienced exmenter provided control experiments are made simultaneously n a standard product of known value. The technic of vitamin D ys has been thoroughly discussed by Coward ('38), who also pros a select bibliography of original literature.

The "Line Test" for Antiricketic Substances: In the ricketic e, growth is proceeding in the sense that cartilage cells are iferating and undergoing metaplasia into osteoid tissue. But caln phosphate is not deposited as in the normal bone. The al ossification in the normal growing bone is in the intercellular rix of cartilage cells, on the diaphyseal (shaft) border of the aphysis. Schmorl ('09) showed that the first sign of healing, the bones of ricketic children, was the deposition of calcium s in this matrix, which he termed the "provisional zone of calcifica-."

The Baltimore group in its studies of experimental rickets sought hake use of the observation of Schmorl as a test for the antiricketic stance. Since rickets in infants or animals may be of various degrees everity, and in its milder forms the bones may show some degree calcification, it was essential for the development of the desired ogical test to discover a diet which would produce without fail young rats florid rickets in which the bones would be free from s showing ossification. The provision of vitamin D would then ate the normal process of calcification and the amount of such ification in a given period of time could be used as a basis of mating the amount of the vitamin administered. Examination of bones of rats restricted to many dietary formulae showed that these ditions were met by Diet 3143, the composition of which has been n.

When the ricketic diet (No. 3143) was fed to young rats they deped acute rickets in 19-21 days. When a source of vitamin D was vided during 6 to 8 days there appeared in the ricketic metaphysis, ght angles to the axis of the shaft of the bones, a honey comb-like osit of calcium salts in the intercellular matrix of the cartilage cells. atment of a split section of the bone with silver nitrate and exure to light caused a blackening of the newly formed calcified area

which could be easily seen in sufficient detail under low magnificati This test is still the basis of most bioassays for vitamin D.

Composition of Blood: Howland and Kramer ('21) had poin out that during active rickets in children the inorganic phosphorus the blood serum is reduced. Since then investigators of this deficie disease have been interested in the use of this criterion for diagnosi rickets. The most extended experimental work with animals has gi results in most respects similar to those reported for children. Ricke rats, especially those on the usual high-calcium, low-phosphorus die like ricketic children, have a normal or slightly low blood calcium, a a decidedly low content of inorganic phosphate. In case of expe ments with rats, this method has the disadvantage that such anima being small have so little blood in their bodies that even with mic methods for the determination of calcium and phosphorus, the chang in blood composition must be followed not on a single rat, but o groups, each group being maintained under the same conditions experiment.

The X-ray Method: Bourdillon, Bruce, Fischmann and Webste ('31) worked out the details of this procedure and used this metho extensively in their work. The bones of the ricketic rats or childre are examined before administration of vitamin D and after 14 day of vitamin therapy. The X-ray photograph gives a picture of healin bone similar to that given by the line test, except that the latter show a section of the layer of healing, whereas the X-ray picture shows th whole thickness of the zone of healing.

Serum Phosphatase as an Index of Rickets: As has already bee stated, rickets can be diagnosed with certainty only by a histologica examination of the bones which, while very valuable in experimenta studies on animals, is of no use in clinical work. There is genera agreement that X-ray signs frequently fail to parallel clinical signs Barnes and Carpenter ('37) have pointed out that one worker may b able to check his own X-ray reading, but that there is much variatio when different individuals read the plates, even though all are ex perienced.

It is relatively safe to assume that an infant with a serum inorganic phosphorus value of only 4 mg. per 100 cc. of serum, or less, has active rickets. The converse of this, namely, the conclusion that the finding of a normal serum phosphorus content indicates that the infant does not have rickets, is often false. It is equally misleading to assume that because the serum phosphorus of an infant has risen from 4 mg. per cent the disease has been cured. The serum phosphorus responds with great rapidity to antiricketic treatment.

A more recently developed diagnostic measure is the blood serum level of the enzyme phosphatase. Kay ('30) reported that this enzyme

ich hydrolyses certain phosphoric acid esters, is abnormally high the serum of ricketic infants. Bodansky and Jaffe ('34) concluded t the level of serum phosphatase is an excellent index to the vity of rickets, and is also a measure of the rate of healing. Barnes l Carpenter (l.c.), using Bodansky and Jaffe's technic, determined enzyme in the sera of 187 infants in Detroit. According to X-ray gnosis, only 50, or 26.7 per cent, had active rickets, and only 37, 19.8 per cent, showed a serum inorganic phosphorus level below ng. per cent. By the criterion of the phosphatase of the serum, how-r, 123 of the 187 infants, or 65.8 per cent of the group, had active kets. Thus the serum phosphatase level is in much closer agreement h the clinical findings than the results obtained by X-ray or blood um inorganic phosphorus determinations.

The Bone Ash Method: In this method the percentage of ash in fat-extracted dry bone is determined. This indicates the absolute cification of the bone. This is one of the most accurate means for antitatively determining the vitamin D content of a substance. It a laborious and time consuming method.

Determination of Vitamin D by Means of Chicks. The X-ray hnic and the "line test" as used on rats cannot be used on chickens, healing of chicks does not show itself as a line of calcification oss the metaphysis in the cut bone. In this species healing is physeal. Consequently the criterion used in measuring calcification chicks is the ash content of the bones.

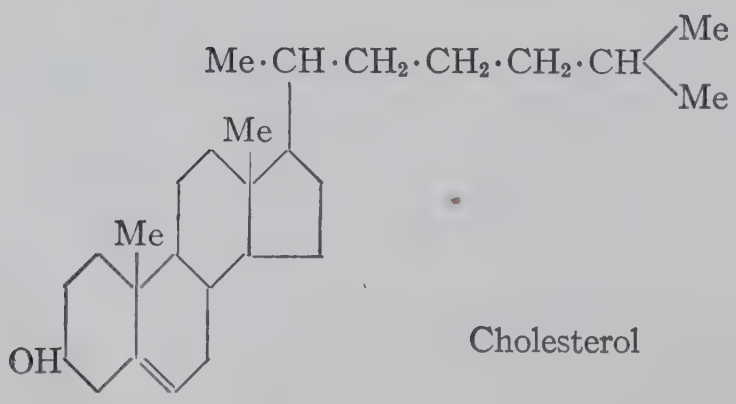
The Unit of Vitamin D.—In the earlier application of bio-assays, eral investigators proposed units for vitamin D, and the result was eat confusion. In 1934 the International Unit, defined by a Com-ttee of the League of Nations, was adopted for the U. S. Pharma-poeia, and also for the New and Non-official Remedies of The Amer-n Medical Association. This unit has now replaced all of the older its. A list of equivalents used by the earlier investigators is pre-ted, in comparison with the International Unit, for the benefit of se who may wish to interpret earlier data. This list expresses the tency of average cod liver oil of medicinal grade.

International Units	100 per gram
U.S.P.10th Ed. Units	100 " "
British, of Medical Research Council Units	100 " "
Steenbock Units	37 " "
American Drug Manufacturer's Units	350 " "
Oslo, or Poulsson (1928) Units	110 " "
Oslo, or Poulsson (1933) Units	160 " "
German Units (rat)	15 per cc.
German Units (clinical)	0.15 per cc.
Cod Liver Oil Coefficient (New and Non-official Remedies)	2.8 D
Cod Liver Oil Coefficient, true	1.0 X

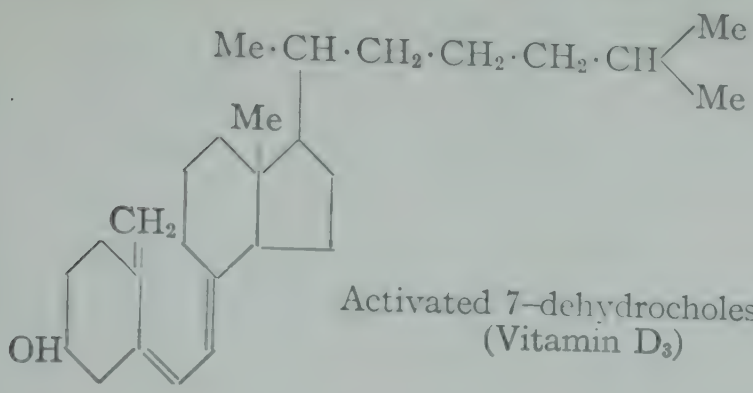
On the basis of the above values, a solution containing 1,000, I. U. per gram of irradiated ergosterol (calciferol) is 10,000 times potent as average cod liver oil. This would formerly have been labe 10,000 X or 28,000 D, and would have been equivalent to 370,000 Ste bock Units per gram.

Biochemical Relation to Vitamin D, Sex Hormones, and Ca cerigenic Substances.—The relation of the sterols, especially vitam D, the sex hormones, and cancerigenic substances is noteworthy this point. The investigations on the structure of the various forms vitamin D and the sex hormones have stimulated the production, cha acterization, and degradation of the sterols so that the structural rel tionships between certain of these groups are now well establishe The carbon skeleton common to these compounds consists of a co densed isocyclic system composed of 3 six-membered and 1 fiv membered rings. The sex hormones are products with the identica tetracyclic carbon skeleton, but with modified side chains. The onl difference in constitution between the sex hormones and the vitamin D is in the long side chain common to the latter which is attached t the five-membered ring. Cholesterol is believed to be the single paren substance from which all of the sex hormones may be derived. The stages of the transformation consist of the total or partial degradation of the side chain resulting in the formation of the unsaturated ketones and then the oxidation of the hydroxyl group.

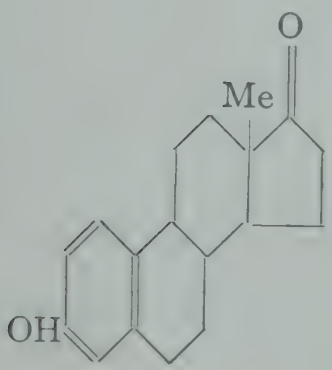
Another group of hydrocarbons whose molecular structure bears a certain resemblance to the sterols is the cancerigenic compounds which also exert a noticeable estrogenic action. It is thought that the mode of formation of these substances is as follows: dehydrogenation of sterols forming the estrogenic hormones and the further dehydrogenation gives rise to cancerigenic compounds having the phenanthrene nucleus, as for example, methyl cholanthrene.



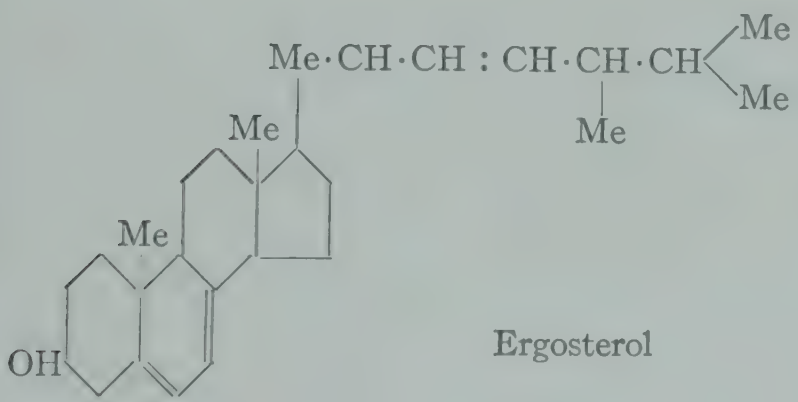
Cholesterol



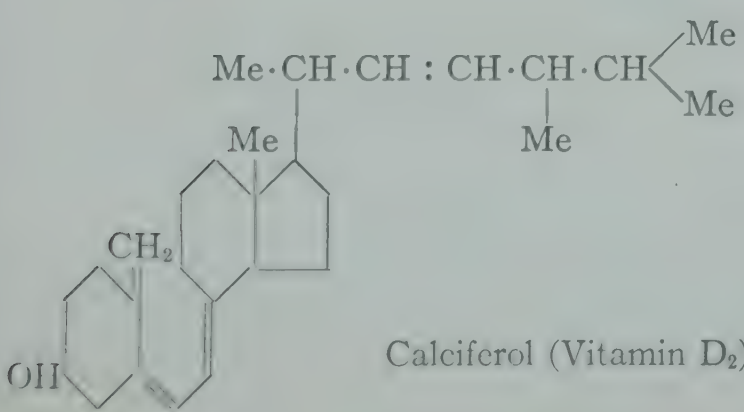
Activated 7–dehydrocholesterol
(Vitamin D_3)



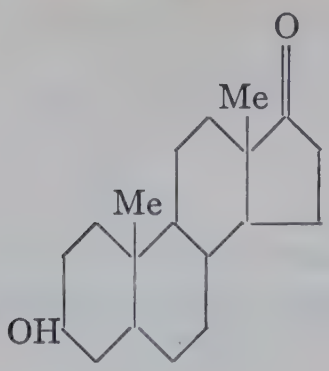
Estrone



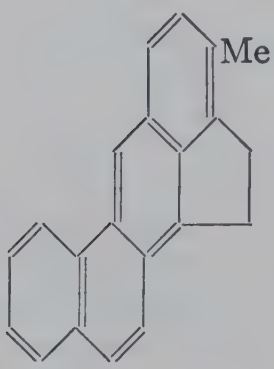
Ergosterol



Calciferol (Vitamin D_2)



Androsterone



Methylcholanthrene

The relation between the sterols, vitamins D, sex hormones, cancerigenic substances is emphasized by the discovery that ergoste calciferol, and other sterols, as well as some of the cancerigenic hy carbons, 1:2 benzpyrene and 5:6 cyclopenteno-1:2-benzanthracene, example, were found to have definite estrogenic activity. Still m important is the newly found correlation between estrogenic and c cerigenic properties. Thus a single molecule may have two enti different effects, such as the cancerigenic and estrogenic proper or the antiricketic and estrogenic properties. The question ari therefore, as to whether metabolic substances originating from vitamins D or sex hormones may not be concerned in the product of malignant tumors.

REFERENCES

Howland (1920). Nelson's Loose Leaf Living Med., *3*, 127.
Mellanby (1918). Lancet, *ii*, 767.
Korenchevsky (1922). Med. Res., Com. Spec. Rep., No. 71.
Goldblatt (1923). Biochem. J., *17*, 298.
Hess and Unger (1920). J. Am. Med. Assoc., *74*, 217.
Sherman and Pappenheimer (1921). Proc. Soc. Exper. Biol. & Med., 193.

EY, PARK, MCCOLLUM and SIMMONDS (1922). Am. J. Dis. Child., *23*.

EY, PARK, MCCOLLUM, SIMMONDS and PARSONS (1921). J. Biol. Chem., 343.

LLUM, SIMMONDS, BECKER and SHIPLEY (1922). J. Biol. Chem., *53*,

(1923). Physiol. Rev., *3*, 106.
(1890). The Practitioner, *45*, 270.
SCHINSKY (1919). Deutsche med. Wchnschr., *45*, 712.
EY, PARK, POWERS, MCCOLLUM and SIMMONDS (1921). Proc. Soc. er. Biol. & Med., *19*, 43.
(1924). Trans. Am. Ped. Soc., *36*, 57; Am. J. Dis. Child., *28*, 256.
BOCK (1924). Science, *60*, 224.
PAPPENHEIMER and WEINSTOCK (1922). Proc. Soc. Exper. Biol. & l., *20*, 14.
WEINSTOCK and HELMAN (1925). J. Biol. Chem., *63*, 305.
BOCK and BLACK (1925). J. Biol. Chem., *63*, 25.
(1935). Physiol. Rev., *15*, 1.
(1938). J. Am. Med. Assoc., *110*, 2150.
TZ and MORSE (1925). Am. J. Dis. Child., *30*, 199.
HEIM and WEBSTER (1926). J. Soc. Chem. Ind., *45*, 932.
RON, KAMM and MORTON (1926). J. Soc. Chem. Ind., *45*, 932.
(1926). Nachr. das. Wissensch. Goettingen, Math.-physik. Klasse, 142.
(1927). Nachr. das. Wissensch. Goettingen, Math.-physik. Klasse, 185.
AUS and HESS (1927). Nachr. das. Wissensch. Goettingen, Math.-sik. Klasse, 175.
HEIM and WEBSTER (1927). Lancet, *i*, 306; Biochem. J., *21*, 389.
HONEYWELL and MACNAIR (1928). J. Biol. Chem., *76*, 251.
HL and ACKERSON (1930). Poultry Sci., *9*, 334.
NGALE and NUSSMEIER (1930). J. Biol. Chem., *87*, 423.
E, RECORD and KENNARD (1933). J. Nutr., *6*, 413.
ELL (1934). J. Biol. Chem., *105*, 711.
AUS, LETTRÉ, and SCHENCK (1935). Liebig's Ann., *520*, 98.
, BOURDILLON, BRUCE, JENKINS and WEBSTER (1930). Proc. Roy. Soc., *97*, 76.
, BOURDILLON, BRUCE, CALLOW, PHILPOT and WEBSTER (1932). Proc. Soc., *B 109*, 488.
, ASKEW, BOURDILLON, BRUCE, CALLOW, FISCHMANN, PHILPOT and BSTER (1931). Proc. Roy. Soc., *B 103*, 340.
AUS (1931). Proc. Roy. Soc., *B 108*, 568.
AUS, LÜTTRINGHAUS and DEPPE (1931). Ann. *489*, 252.
ON (1927). Science, *66*, 176.
ON and COOLIDGE (1927). Proc. Soc. Exper. Biol. & Med., *24*, 366.
ON and MOORE (1929). J. Biol. Chem., *81*, 49.
AN and DANIELS (1936). J. Biol. Chem., *115*, 119.
ARRIE, THOMPSON, STOESSER and RIGLER (1937). J. Pediat., *10*, 295.
, MUND, POURBAIX and CASTILL (1930). Comp. rend. Soc. de biol., 534.

Windaus and Bock (1937a). Ztschr. f. physiol. Chem., *245*, 168.
Boer, Reerink, van Wijk and van Niekerk (1936). Proc. Acad. Sc. sterdam, *39*, 622.
Windaus and Bock (1937b). Ztschr. f. physiol. Chem., *250*, 258.
Bills (1926). J. Biol. Chem., *67*, 753.
Yoder (1936). J. Biol. Chem., *116*, 71.
McDonald (1937). J. Biol. Chem., *114*, lxv.
Stavely and Bergman (1937). J. Org. Chem., *1*, 567.
Weinhause and Kharasch (1936). J. Org. Chem., *1*, 490.
Windaus and Trautman (1937). Ztschr. f. physiol. Chem., *247*, 185.
Bills, Massengale, Hickman and Gray (1938). Proc. Am. Soc. Chem., *8*, No. 7, x.
Belloc, Fabre and Simonnet (1930). Compt. rend., *191*, 160.
Copping (1934). Biochem. J., *28*, 1516.
Leigh-Clare (1927). Biochem. J., *21*, 368.
Drummond and Gunther (1930). Nature, *126*, 398.
Darby and Clarke (1937). Science, *85*, 318.
Schoenheimer (1931). Science, *74*, 579; J. Biol. Chem., *92*, v.
Daniel and Munsell (1937). U. S. Dept. Agr., Mis. Pub., 275.
DeVaney, Munsell *a*nd Titus (1933). Poultry Sci., *12*, 215.
Drake (cited by Park, 1938). J. Am. Med. Assoc., *111*, 1179.
Campion, Henry, Kon and Mackintosh (1937). Biochem. J., *31*, 81.
Bechtel and Hoppert (1936). J. Nutr., *11*, 537.
Report of the Council on Foods (1937). J. Am. Med. Assoc., *108*, 206.
Coward (1938). "The Biological Standardisation of the Vitamins." Billie Tindall & Cox, London.
Schmorl (1909). Ergeb. der Inn. Med. u. Kinderh., *4*, 403.
Howland and Kramer (1921). Am. J. Dis. Child., *22*, 105.
Bourdillon, Bruce, Fischmann and Webster (1931). Med. Res. Coun Special Rep. Series, No. 158.
Barnes and Carpenter (1937). J. Pediat., *10*, 596.
Kay (1930). J. Biol. Chem., *89*, 249.
Bodansky and Jaffe (1934). J. Biol. Chem., *105*, xi-xii.

CHAPTER

XV

Significance of Vitamin D in Rickets and Related Diseases

RESEARCH accumulates it becomes increasingly evident that the icketic substances are concerned with physiological processes other calcification of growing bone. This chapter will be devoted to iscussion of various aspects of vitamin D in nutrition, which in- the present concepts regarding its mode of action in rickets, elationship to the state of calcium and phosphorus in the blood to the dietary content of these minerals, storage, absorption and tion, species differences in utilization of the various forms of in D, normal requirement and distribution of this factor, the of vitamin D in diseases related to rickets, and the therapeutic of this substance.

tructure of Normal Bone.—The growth of normal bone at the ion of the epiphysis, or head, with the diaphysis, or shaft, is pre- d by the following changes in the cartilage of the epiphysis: he cells of the indifferent or resting cartilage become enlarged arrange themselves into columns of four to six cells, and become nted by mutual pressure so as to present, in cross section, a roughly ngular shape. This forms what is known as the proliferative zone rtilage. Near the shaftward end of this proliferative zone the um is deposited in the intercellular substance of the cartilage, ing the so-called zone of provisional calcification which abuts ne end of the growing shaft in a curved or straight, but always -cut regular line. Each cartilage cell is enclosed in a capsule of ied intercellular matrix. The shaft grows at the expense of the aginous epiphysis. Each calcified cell capsule is perforated by a e advancing capillary loop from the marrow cavity. The presence ne blood supply thus brought to the cartilage cell causes it to rgo dissolution. The capillary loop advances to the next inter-

cellular matrix wall and again liberates and destroys the neighbori cartilage cell. During this process of destruction of cartilage on t shaft end of the cartilage disc, new swollen cartilage cells are arisi on the epiphyseal end, so the number of cells in this state remain m or less constant at four to six in a column. The calcified intercellu substance which separates the cells in the direction parallel to t long axis of the bone is not attacked by the blood vessels, and rema imbedded in the advancing shaft as fine lines thickened at the poi where the intercellular walls running at right angles to the long a of the shaft were destroyed. Osteoblasts which have advanced from t marrow along with the blood vessels attach themselves to these spicu of calcified intercellular substance and surround themselves wi osteoid tissue (bone tissue without a deposit of calcium salts); this speedily converted into bone by the deposition of calcium salts, chie calcium phosphate. New osteoblasts continue to settle on the growi bone trabeculae and the process described is repeated, resulting in the enlargement.

Structure of Bone in Rickets.—In ricketic bones a provision zone of calcification is not formed, since lime salts fail to be deposite in the intercellular substance of the cartilage. The proliferative ca tilage persists in some places, and in others is destroyed in an irregul way by aberrant blood capillaries which sprout in a disorderly fashio from the parent vessels in the shaft and invade the cartilage in all d rections. It is for this reason that the line of junction of the epiphysi and the shaft becomes very irregular and long tongues and islands o cartilage are left isolated. Either the cartilage cells undergo metaplasi into osteoblasts or osteoblasts follow the blood vessels as they invad the cartilage and form osteoid tissue which, however, does not receiv a deposit of lime salts. Lack of calcification results in lack of rigidit of the bones, and the body apparently attempts, unsuccessfully, to com pensate for this weakness by producing osteoid tissue in excessiv amounts, hence the enlargement of the ends of the bones in rickets Because of the abnormal growth at the end of the shaft, a zone know as the ricketic metaphysis is formed, which consists of connectiv tissue, osteoid tissue, marrow elements, blood vessels, and cartilage cell in all stages of degeneration and metaplasia into osteoid substance The directive influence exercised by calcification of the intercellula substance in cartilage being absent, all other growth phenomena i the growing bone become aberrant, and the anatomical elements be come disarranged.

Immediately upon the administration of vitamin D in sufficien amount, the content of ionized calcium and of inorganic phosphate i the blood, which may fall below the normal level in subjects in the ricketic state, tends to return toward normal values. There is at pres

no way to decide whether the solubility product of calcium and osphate ions ($Ca^{++} \times PO_4^{\equiv}$), expressed in milligrams of calcium d of inorganic phosphate per 100 cc. of blood, alone determines the osition of lime salts in the intercellular substance (provisional cification), or whether, in addition, there is a conditioning of the s of the cartilage which brings about this deposition, or whether h effects are involved. The fact is that provisional calcification im-diately begins and with this the directive processes leading to rmal bone growth are established. Those tissue elements in the ketic metaphysis which are in disarray are quickly cleared away, d the histological appearance of sections of the bone show that rmal bone growth has been resumed.

The Etiology of Rickets.—Rickets was established as a definite ease in 1650. Little was known about the exact nature of the disease il Pommer in 1885 made his extensive studies of ricketic bones. 1890 Palm reported the results of a geographic investigation of this ease and pointed out the beneficial effect of sunlight in its treat-nt. Buchholz ('04) first called attention to the value of treatment of kets with artificial light; his observations, however, attracted but le notice. In 1919 the etiology of rickets was still a mystery. At this ne Huldschinsky ('19-20) reported on the curative action of ultra-let rays from a mercury vapor quartz lamp, and Hess and Unger 0-21) confirmed his findings.

Contemporaneously with the studies on sunlight in relation to kets, another group of investigators followed the theory that rickets s the result of a faulty diet. The work of Mellanby ('19) gave great petus to the study of experimental rickets. Mellanby ('21) studied kets in puppies about two months of age. Having formulated a t consisting of skim milk, bread, linseed oil, yeast, orange juice, and lium chloride, which regularly produced rickets in pups, he supple-nted it with various kinds of fats and noted their effects on the letons. His pups were in most cases kept indoors, but some were sely confined, some were kept out-of-doors, or allowed to run in enclosure. As criteria of rickets he employed the appearance, X-ray dings, content of calcium in the bones, and histological appear-ce of the bones. He found cod liver oil to be very effective in eventing abnormalities of the bones. Suet and butter fat had con-erable antiricketic value, while lard had none. Peanut oil was ter than coconut oil; rapeseed, cottonseed, palm kernel, olive, and seed oils had little or no value, and babassu oil was considered e "worst."

Hopkins (1920) was the first to show that if oxygen passed through ated butter fat, vitamin A is readily destroyed. In consequence, Mel-by ('21, l.c.) compared the antiricketic effect of heated and unheated

butter and found that the oxidized fat did not prevent rickets in pu
However, oxidized cod liver oil retained the antiricketic effect. He
undecided "Whether this difference . . . can be simply explained
the fact that cod-liver oil contains a much greater quantity of a
rachitic vitamine than butter, so that the destructive change take
longer time, or whether some other explanation must be sought."
found that lean meat had an antiricketic action sufficient to preve
or limit the severity of rickets under favorable circumstances, b
under unfavorable conditions it did not prevent the disease. The high
the content of bread in the diet, the more severe was the ricke
Casein containing some calcium prevented rickets, but casein pr
pared by precipitation with hydrochloric acid and free from calciu
seemed to intensify the rickets in pups. The addition of tricalciu
phosphate to the diet did not prevent the disease. Mellanby employe
the term "antirachitic vitamine" and expressed the belief that whe
the diet is well constituted a small amount of the vitamin woul
suffice for protection; if badly constituted, in a ricketic sense, a large
amount would be necessary. He found that older animals were les
susceptible to rickets, and had less need for the antiricketic vitamin
Also, it was his belief that "osteporosis" in dogs may give rise t
deformities somewhat identical with those seen in rickets. He stressed
environmental conditions and confinement as etiological factors in
rickets. The composition of the inorganic moiety of the diet was no
taken into consideration. More extensive histological studies would
have made these experiments more convincing, since it is generally
agreed that gross lesions of deformity resembling closely those seen
in rickets may result from other causes. The ricketic bone has the
following characteristics: (1) calcification of the provisional zone of
cartilage (intercellular substance) does not occur; (2) cartilage cells
persist in the shaft below the normal epiphyseal line; (3) there is
irregular invasion of the cartilage by blood vessels and connective tis-
sues; (4) newly formed preosseous tissue remains uncalcified and is
produced in abnormal amounts—the so-called osteoid tissue; (5) there
is developed the ricketic metaphysis.

The investigation of the etiology of rickets by McCollum, Sim-
monds, Parsons, Shipley and Park ('21) was undertaken with the
criteria just mentioned as the basis of judgment concerning the effects
of numerous diets on the skeletal development of young rats. Park
and Shipley were skilled in the histology of the bones. An examina-
tion of the bones of rats kept during several weeks on each of several
hundred modifications of diet, in which all the known factors were
systematically varied, the animals being kept indoors, showed that two
dietary characteristics were of outstanding significance in the etiology
of rickets, resembling in all details that seen in infants. These were the

o between calcium and phosphorus and the presence or absence of ain fats in the diet.

The observation of Mellanby concerning the antiricketic value of liver oil, and the much smaller value of butter fat, was confirmed. vas found that when a diet, devoid of an antiricketic fat, provided ut 0.20 gm. of phosphorus and 1.2 gm. of calcium per 100 gm. of d, acute and severe rickets developed in young rats in 19 to 21 s. Diets containing two to three times this amount of phosphorus, half as much calcium, were not effective in producing rickets in rat, an animal which is rather resistant to the disease. In the latter the bones were not entirely normal in histological structure, but vas evident that a wide ratio of these elements was of fundamental portance and inimical to the normal development of the animals. h the wide ratio of P/Ca of 0.20:1.2, the regulatory mechanism one development failed entirely; but the provision of an anti- etic fat furnished the necessary regulatory effect, and the bones e normal in structure.

When the diet provided 0.043 per cent of calcium and 1.2 per cent phosphorus, rickets also developed, but it was complicated by ny. Analysis of the blood plasma of ricketic rats on the high-calcium -phosphorus diet showed that the calcium content was normal and phosphorus content low. With the low-calcium high-phosphorus s both the calcium and phosphate values were low.

t was further shown (McCollum, et al., '22) that the feeding of lized cod liver oil, which had been prepared by blowing hot air ugh it to destroy vitamin A, afforded protection against rickets xperimental rats. The cod liver oil was tested and found to be pable of protecting rats against the xerophthalmia characteristic of min A deficiency. Hence it was concluded that another fat-soluble min existed, and that it was abundant in cod liver oil, and much abundant in butter fat, and all but absent in various vegetable This new factor was designated vitamin D.

n 1921 Sherman and Pappenheimer published an important paper ch described the production of rickets by means of a diet low in sphorus, and its prevention by the addition to the diet of alkaline ssium phosphate. Their diet was composed of white flour 95 per , calcium lactate 3 per cent, sodium chloride 2 per cent, with or out the addition of a trace of ferric citrate. When 0.4 per cent he potassium phosphate was added and an equivalent amount of um lactate withdrawn, the animals were completely protected nst rickets. These investigators, therefore, demonstrated independ- y of McCollum and associates the profound importance of a er balance of calcium and phosphorus in the diet for the develop- t of normal bones. They did not study the influence of fats on the

etiology of rickets. Their diet was lacking in vitamin A and all th water-soluble vitamins, as well as in vitamin D, but rickets develope so promptly that the other deficiencies did not have time to vitia their experiment. Soon afterwards Pappenheimer, McCann, Zucke and Hess ('21-22) showed that addition of small amounts of yeas orange juice, butter fat, and potassium, did not exert a protective in fluence on the animals.

It is clear that when the nutritional state is adequate as respects a other factors, the normal calcification processes involving the osseou tissues are chiefly dependent upon the calcium and phosphorus ratio in the food and upon an adequate supply of vitamin D. The mechan ism is by no means so simple as this, however. Shohl and associate ('32) have demonstrated that when the content in the diet of both calcium and phosphorus is greatly increased, the ratio being as wide a in the rickets-producing diets, the development of rickets is prevented When the total intake of these elements is lowered, the rickets-produc ing quality of the diet is increased when the Ca:P ratio is unfavorable Thus, at each level of calcium a different ratio is necessary to produce rickets, as measured by X-ray, histology, blood serum calcium and phosphorus, and bone ash. There is, therefore, a degree of protection afforded against rickets by the feeding of favorable ratios of calcium and phosphorus and of both elements in amounts which provide them in abundance. Excellent discussions of this subject have been written by Park ('23) and Hess ('29).

Gross Anatomical Changes in Rickets.—In the long bones the main deformities are due to bending. When recovery occurs, the deformities are fixed, with consequent impairment of bodily mechanics. The ribs are enlarged at the junction of the rib and sternal cartilage, forming the ricketic rosary. Deformity of the thorax may result, in severe cases, in the so-called "chicken-breast," or the "funnel-shaped chest." Changes in the shape of the thorax together with softness of the ribs impairs breathing, and the enlarged costo-chondral junctions may press from the underlying portions of the lungs causing collapse in these areas, and in turn, compensatory emphysematous areas may form along the anterior borders. Pneumonia is not infrequently a complication of moderate or severe rickets, which causes a marked susceptibility to respiratory infection.

The spinal column and the vertebrae are usually not deformed, except in severe cases, when the column may be curved as in scoliosis or kyphosis or both. The vertebrae are abnormally soft, the intervertebral discs varying in width, and the ligaments are flabby. The cranium shows characteristic changes, the fontanel tending to be abnormally wide, and deformities of the skull result from softness of the bones along the suture lines. The frontal bones may be greatly hypertrophied,

ng rise to the "Olympian front" which is characteristic of ets.

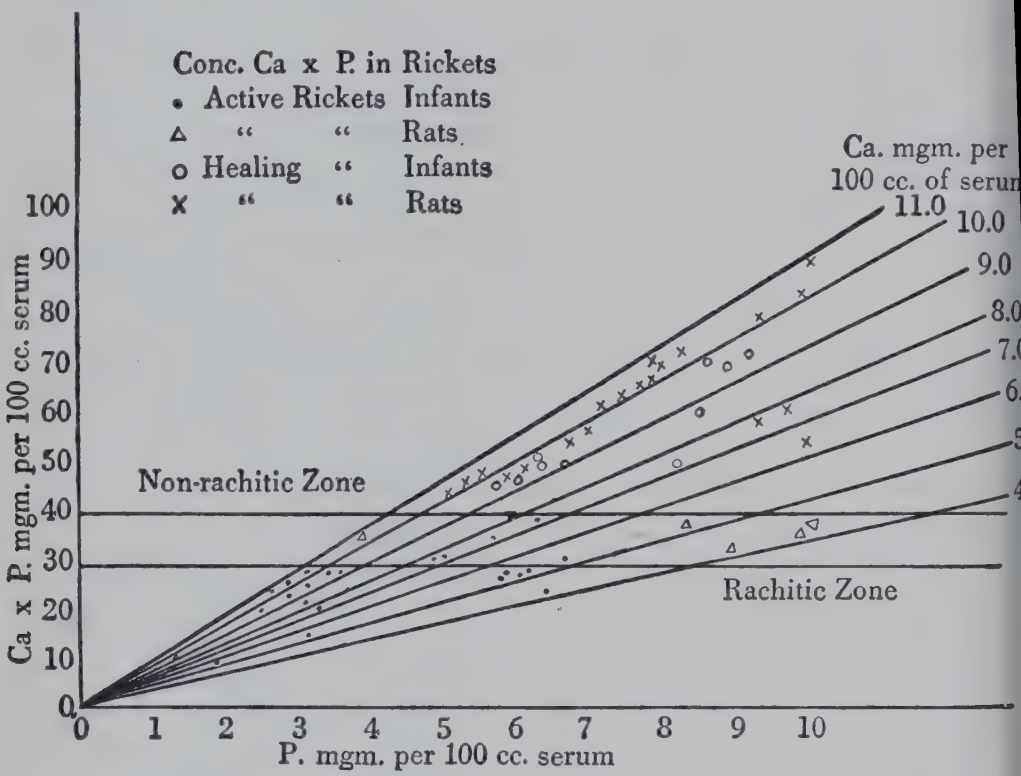
Cretinism, chondrodystrophy, osteogenesis imperfecta, and scurvy, also characterized by bone deformities. However, they are entirely inct from those seen in rickets.

The Chemical Composition of the Blood in Rickets.—Howland Kramer ('21), from their studies of the inorganic phosphorus of blood serum of both normal and ricketic children, concluded that ganic phosphate represents a definite chemical entity in the blood, e it is present in nearly constant amounts in normal individuals of same age. In rickets, there was a marked decrease in inorganic sphorus of the serum, and to this deficiency they ascribed the fail- of deposition of calcium salts in the bones. The blood serum of -ricketic infants and young children contained between 10 and 11 of calcium and about 5 mg. of inorganic phosphorus per 100 gm. children under two and a half years with 3 or less than 3 mg. of sphorus had active rickets. When cod liver oil was administered phosphorus content of the serum returned to normal, and the rise he phosphate of the serum was accompanied by healing of the etic lesions. Kramer and associates ('21) found the serum calcium ases of rickets associated with tetany to be about 5.4 mg., or roughly the normal. They found the inorganic phosphate of the sera of e infants who had rickets associated with tetany to be within the nal limits, or slightly above normal.

n rats, Kramer and Howland ('22) found the concentration of m calcium to be 9.5 to 10.5 mg. and of phosphorus from 7 to mg. per 100 cc. These values are not increased by changes in the or by ultraviolet light. In animals fed diets somewhat low in um or phosphorus the content of these elements in the serum d be diminished by as much as 50 per cent. On the same diets the ision of cod liver oil brought the values back to normal. They d that irradiation of the animals with ultraviolet rays likewise ed the phosphorus content of the serum, as did also a few days tarvation. Brown and coworkers ('22) reported a study of two st-fed infants with rickets accompanied by tetany. The mothers' s were of very poor quality. The milks of both women were ab- nal in their ash contents, one being low in calcium and high in sphorus, the other was low in both calcium and phosphorus. The d sera of both infants were low in calcium and phosphorus.

Howland and Kramer ('22, '23) showed that in uncomplicated ets the concentration of calcium in the serum was normal or nearly whereas that of the phosphorus was regularly low, resulting in a luct of the two concentrations, expressed in milligrams per 100 cc. r below 30. In tetany the product was also low on account of the

striking reduction of the calcium content. After giving cod liver
or ultraviolet therapy, there was such a great rise in inorganic ph
phorus as to make the product two or three times the one bef
treatment. The following graph taken from their paper was c
structed from data obtained from the analysis of sera of both child
and rats. These experimenters interpreted the results shown in t
graph to indicate that when the Ca x P product is 30 or below, rick
is to be expected; and when the product is 40, it is possible to ha
rickets. When it is above 40, either healing is taking place or rick
is absent.



Graph taken from paper by Howland and Kramer, Monatschr. f. Kinderheilkund
1923, xxv, 279.

Since these findings were reported, numerous experiments hav
demonstrated the complexity of the calcium and phosphorus in th
blood, consequently this criterion of diagnosing rickets cannot be re
garded as representing a simple chemical state conforming to the law
of solubility product (Hess et al., '30; McGowan, '33).

The blood phosphatase and bone phosphatase are markedly in
creased in rickets. Phosphatase is an enzyme which hydrolyzes certai
organic compounds of phosphorus and liberates the element as inor
ganic phosphate. Recovery from rickets is indicated by a slow decreas
in serum phosphatase. In normal children the phosphatase content o
blood ranges from 5 to 14 Bodansky units per 100 cc. The measure

of this enzyme serves as an aid in diagnosis and treatment of
ninosis D.

e Effect of the Ratio of Calcium to Phosphorus in the Diet on Content of These Elements in the Blood Serum.—Kramer and and ('32) analyzed the blood serum of rats prepared by Mc-m and Simmonds for the purpose of showing the influence of alcium and phosphorus content and of vitamin D on the con-tion of these elements in the blood serum. The diet employed ted of rolled oats 40, gelatin 10, wheat gluten 7, NaCl 1, KCl 1, extrin 41 gm., respectively. The proportion of calcium to phos-s was varied by adding $CaCO_3$ or KH_2PO_4, or both. The ani-were restricted to the diets for about 6 weeks. The calcium of the vas varied from 0.14 to 1.24, and the phosphorus from 0.2 to 1.2 er 100 gm. of food. Vitamin D was provided by 1 per cent and r cent of butter fat, and by 2 per cent of cod liver oil. These nts of the fats provided very low, low, and liberal amounts of tamin. Typical results are shown in the following table:

EFFECT OF VARIABLE VITAMIN D IN DIET

TAMIN D	SERUM Ca	SERUM P	WEIGHT GAIN, GM.
Diet Ca/P=0.4	P=1.0	Ca=0.44	
.F. 1	6.3	10.4	7.0
.F. 10	10.7	9.9	43
.L.O. 2	9.8	7.6	70
Diet Ca/P=1.0	P=1.0	Ca=1.04	
.F. 1	8.0	8.0	20
.F. 10	10.7	8.8	55
.L.O. 2	10.3	8.3	68
Diet Ca/P=1.5	P=0.8	Ca=1.24	
.F. 1	10.5	8.7	23
.F. 10	10.5	8.8	55
.L.O. 2	10.2	8.5	82
Diet Ca/P=6.0	P=0.2	Ca=1.24	
.F. 1	10.2	3.0	20
.F. 10	11.2	3.9	12
.L.O. 2	13.6	7.5	10

The maintenance of a normal concentration of calcium an ganic phosphorus in the serum is an example of biological reg Other examples are the maintenance of a nearly constant boc perature in warm-blooded animals, of a fixed hydrogen ion c tration in the blood, and of a constant osmolar concentration serum. Various factors such as the concentration of calcium an phorus in the diet, vitamin D, protein, fat, carbohydrates, vario other than calcium and phosphates, and the balance between ac basic ions, all play a part in determining the absorption of bon ing salts.

The type of phosphorus in the diet is also of great impo In the case of the phosphorus of cereals, for example, it was that their "anticalcifying" effect was due to the presence of phorus in the form of inositolphosphoric acid which is poorly a able. The biological availability of the phosphorus from di sources has been discussed in detail in Chapter VII. Endocrine may influence the ebb and flow of calcium salts from and in tissues and their excretion from the body.

If the intake of calcium is low and that of phosphorus is hig excess of phosphorus which is absorbed is excreted, and it a that, notwithstanding the needs of the body for calcium, the phate ion carries with it some calcium, thus depleting the blood ionized calcium. When the situation is reversed, phosphate ion carried away with the excess of calcium excreted, even thoug be to the disadvantage of the body. When young rats are caus develop acute rickets by restricting them to a high-phosphorus calcium diet, the blood calcium is lowered to a level which pro tetany as an accompaniment of rickets. Tetany never compl rickets produced by a high-calcium low-phosphorus diet.

Role of Vitamin D in Mineral Metabolism.—There is a str regulatory power of vitamin D on the calcium and phosp metabolism of the body. The action of the antiricketic vitamin i body is directly concerned in the preservation of the mineral ba Vitamin D not only acts as a regulator of the metabolism of elements; it permits the body to operate with greatly increased omy. When it is present in optimum amount, the metabolism of minerals is maintained in a nearly normal manner even when o both of them is provided at almost starvation levels. The vit brings about maximal utilization of calcium and phosphorus these conditions, both by reducing excretion and by increasing ab tion.

In active rickets there is a diminished retention of calcium phosphorus. Infants (Orr, Holt, Wilkins and Boone, '23) with rickets fail to retain calcium and phosphorus although ade

nts are present in the diet. On irradiation with ultraviolet light elements are readily retained. It is generally assumed that vita-D, either from direct administration or from light acting on the increases the absorption of calcium and phosphorus by the in-. Thus the retention of these minerals returns to normal.

McGowan ('33, l.c.) observed a marked shift in phosphate excre-rom feces to urine after administration of large doses of the n. He observed a definite and consistent rise of the serum phos-s; the serum calcium was unchanged. McGowan believes that m plays a secondary role to phosphorus and is affected only in-h as it acts to neutralize the excretion of phosphate ion.

lzberg and Meyer ('32) noted that in rats on ricketogenic diets, in the deficiency the blood phosphorus decreased more rapidly he calcium. Rominger, Meyer and Bomskov ('32) made similar ations in human rickets and stated that "rickets is a primary dis-nce in phosphorus metabolism." Kay and Guyatt ('33) conclude their experimental studies that rickets is a phosphorus deficiency e. On the basis of their evidence, rickets appears to be primarily phosphorus deficiency, and lack of vitamin D produces rickets creasing the retention of phosphorus which secondarily depletes ganism of calcium; and it is this depletion which is the cause kets.

e experiments of Shohl and Bennett ('27, '28), Karelitz and ('27), Williams ('28), and Warkany ('30), as well as that of y, Kramer and Howland ('26), suggest a close relation between s and a low phosphorus retention. In contrast to these observa-another group of investigators (Murdoch, '27; Courtney et al. och and Cahan, '27) express the belief that the abnormal excre-f phosphorus in rickets may be a result of a change in the cal-metabolism rather than an impaired power of phosphorus ab-on. Daniels and collaborators ('29) also concluded that the im-ce in the amount of calcium and phosphorus retention in rickets more frequently to a deficiency of calcium. Skaar ('27) made r observations; however, he considers that there are two ricketic ses, one where calcium metabolism is affected, the other where horus metabolism is first affected. Cod liver oil therapy causes ood calcium and phosphorus to return to the normal and the e of these mineral elements becomes positive. If phosphorus in rm of Na_2HPO_4 is added to the ricketic diet, the ricketic condi-greatly aggravated and the calcium-phosphorus balances rapidly se. The serum phosphorus is markedly lowered. Skaar and l ('29) conclude that in some individuals the disturbance is con-l with the calcium metabolism and in others with the phos-s.

Vitamin D in Relation to the Parathyroid Glands.—Wilder Howell ('36) reviewing the literature relating to hyperparathyroi have discussed the relation of vitamin D deficiency to parathyroi largement. Deficiency of this vitamin results, among other effects, fall of the ionized calcium of the blood serum. Blood calcium co tration is controlled, in part, by the parathyroid hormone, and in a minosis D there is a compensatory enlargement of the parath glands to offset the deficiency of this nutrient. They state that compensation for deficiency of this vitamin that is effected by hy plasia of the parathyroids is usually not overdone, in that it does exceed the increased need for it. In certain cases, however, adeno formation occurs. The rarity of this condition in man, they sugg is due to the fact that it occurs only in those cases where there embryonic cells, or cell nests, in the parathyroids. In such cases privation of vitamin D, which causes hypertrophy in the gland, le to the proliferation of the embryonic cells, causing parathyroid tum The resulting tumor frequently possesses the power of synthesizing parathyroid hormone. If it does, its function will be without restraint that regulates the normal parathyroid gland, and thus it w provide a supply of the hormone unrelated to the requirements of t body. In this condition an excessive amount of calcium salts will mobilized, the content of calcium in the blood will rise, and excessi amounts will be excreted. On this hypothesis, the etiology of oste malacia, or bone softening, may be tentatively explained. They su gest that in most human subjects there are no embryonic cells in th parathyroids, and that in such subjects, in vitamin D deficiency, th parathyroids are capable of increasing their function without hype trophy. Hamilton and Schwartz ('32) have emphasized the increase function of the parathyroids in children insufficiently provided wit vitamin D.

Wilder and Howell ('36, l.c.) point out that hyperparathyroidism exceedingly rare in sunny climates, and occurs with increasing fr quency at higher latitudes. They correlate this incidence with th amount of sunlight available. In the North Atlantic States 113 cas have been reported per 100,000 of population, as against 9.5 cases i Italy. Hyperparathyroidism may result in osteitis fibrosa. The evidenc seems to be conclusive that deficiency of vitamin D demands com pensatory hormonal production by the parathyroids.

The Antiricketic Activity of Skin, Hair, Wool, and Feathers.— Since vitamin D_3 is formed in the skin through the action of ultra violet light, the effects of irradiating the skin are in all respects com parable with those of the administration of D_2 or D_3 in fish liver oil Eckstein and Wile ('26) found 19 per cent of the total lipids of huma skin to be cholesterol, which constitutes about 1 per cent of the tot

of the skin. Hess and Weinstock ('25) showed by feeding
hat unirradiated skin has no antiricketic value whereas on irra-
it becomes antiricketic. Hair and feathers also are activated by

ol fat contains a provitamin D and is activated on irradiation.
g ('27) reported that ultraviolet rays did not protect rats from
when they were prevented from licking their fur, indicating
ch vitamin D as is formed in their skins is not transferred to
od. But when the hairs are swallowed, the provitamin D in the
dressing derived from the sebaceous glands having been acti-
the vitamin is absorbed. Hou and Tso ('28, '29, '30) state that
in of normal rabbits was slightly antiricketic, the dorsal skin
han the ventral; but that the skin of ricketic rabbits, or of
reared indoors was without protective action. Hou ('30) noted
ter the skin and hair had been washed with ether, ultraviolet
tion was but little effective in preventing rickets. Rowan ('28)
that birds of prey on a meat diet developed rickets, but that they
rotected when fed feathers. Hou ('31) also observed that birds
rom mammals in having but one gland of a sebaceous nature,
e preen gland, which supplies a waxy secretion that the bird
ites upon its feathers for their protection against wetting.
gland oil becomes activated on irradiation. Thus vitamin D is
ed by ingesting feathers, or by absorption from the skin which
from the feathers. The feathers and skin of ricketic birds or
rom which the preen gland had been removed, possessed little
antiricketic property. Removal of the preen gland made birds
ible to rickets, and birds without this gland were not benefited
osure to sunlight or to ultraviolet rays. Nocturnal birds and
rous animals may derive their vitamin D from the fur or
s which they ingest. In line with this view one may explain, in
ne nutritive effect observed from the feeding of rabbits or of
irds, including fur or feathers, respectively, to young Carnivora.
Carnivora have been frequently fed too much muscle meat and
le bone and glandular organs to supply sufficient calcium, phos-
, and vitamins; consequently, their feeding on small animals
may be eaten in their entirety, corrects this difficulty, for the
feathers exert a beneficial effect, being sources of vitamin D.
are said not to thrive if they are frequently scrubbed with soap
ater, which may perhaps be explained on the basis of the im-
ce of the sebaceous secretion upon and in the skin, for absorp-
ter exposure to sunlight. Helmer and Jansen ('37) found that
g removes substances from the human skin which ultraviolet
n convert into an antiricketic agent. Water removes this frac-
uch less effectively than does soap and water. It may be, there-

fore, that persons who are addicted to frequent bathing with th of soap may remove so thoroughly the provitamin and vitamin the skin that they would derive much less benefit from exposu sunlight than would be the case were the washing less thorough. may have a bearing on the question of the need of a dietary sour vitamin D by certain persons during the summer as well as durin winter months.

Hume, Lucas and Smith ('27) found that by anointing the ski ricketic rats with an oil solution of irradiated impure cholesterol, of rickets resulted. Similar conclusions have been reached by Astr and Morgan ('35), Amrhein ('34), and others. This is not surpri since it has long been known that fatty substances are abso through the skin. It is not yet decided whether, when the ski irradiated, vitamin D is formed in the skin, or whether, as Hess, W stock and Helman ('25) suggested, the antiricketic activity of sunli may come about through activation of the provitamin which is creted by the sebaceous glands, and the vitamin then is reabsorb Fodor ('34) concluded that ten times as much of D must be suppl by way of the skin as by mouth in order to initiate the healing rickets in rats. However, it appears that the skin plays a definite r in the absorption of antiricketic substances formed by ultravio irradiation.

Storage.—The animal body maintains a reserve of vitamin D, t concentration varying with the dietary supply and the length of e posure to sunlight or other sources of ultraviolet radiation. The dep tion of this store when the body is not receiving a supply of the vitam is influenced by the calcium and phosphorus content of the diet.

Individual differences in susceptibility to rickets in both expe mental animals and infants have been traced to the diet of the moth during pregnancy and lactation (Hess and Weinstock, '24a; Tover and Toverud, '31). The antiricketic potency of the mother's milk vari with the D content of her diet. A transfer of vitamin D from t maternal organism to the fetus seems to occur. In cases where the die are deficient in vitamin D the bodily storage of D is so low that t young become extremely susceptible to rickets.

From studies with different species it has been shown that D normally present in blood. Heymann ('37) has investigated the fa of this factor within the animal organism and its storage in vario tissues. He demonstrated that upon administration of a single lar dose of viosterol (200,000 U. S. P. units of vitamin D) to male rabbi some of the vitamin remained in the different tissues for vario lengths of time. In the brain it was stored for but 1 to 2 weeks, the red blood corpuscles 5 to 6 weeks, the skin retained it for 6 to weeks, kidneys 6 to 9 weeks, and it remained in the liver and bloo

ıg as 12 weeks. Heymann believes that the time of storage in ver is of significance and agrees with Gerstenberger ('33) that rgan may play a decisive role in the functioning of vitamin D. lso concludes that conditions other than the purely chemical osition of the tissues influence the retention of vitamin D.

ode of Absorption and Excretion of Vitamin D.—Greaves and idt ('33), studying the role of bile in the intestinal absorption amin D, concluded that the vitamin was not absorbed in the ce of bile. Administration of vitamin D failed to cure rickets s with bile fistulas.

eymann ('37a) pointed out that liver injury caused by biliary ıction might also impair the effectiveness of vitamin D. Upon er investigation ('37b and c) he confirmed Greaves' and idt's findings. Heymann suggests that the non-absorption of the in under these circumstances indicates that bile appears to act ıe vitamin D molecule itself. These results substantiate the hesis advanced by Gerstenberger ('33, l.c.) that the action of in D is concerned with some function of the liver. Heymann's al conclusion is that impaired liver function is responsible for the ished effect which vitamin D has upon ricketic rats in which damage had been induced.

studying excretion of the antiricketic factor, Heymann ('37b and found that when bile was excluded from the intestine of dogs, in D was present in their feces for but 10 to 16 days after the istration of large single doses, whereas the normal animal upon r treatment retained the vitamin in the feces from 6 to 8 months. findings indicate that in case of the normal dogs, after about st two weeks following the ingestion of a large single dose, any in D found in the feces must have been absorbed and then excreted.

ansmission of Vitamin D to Milk by Lactating Women.—A er of studies have been made to determine whether taking vita- D by the human mother suffices to protect her breast-fed infant t rickets. Hess and Weinstock ('24b) found that 15 of 28 developed rickets although their mothers took cod liver oil dur- e last two months of pregnancy. Gerstenberger, Hartman and ('27) observed that breast milk from women receiving one oonful of cod liver oil daily, failed to cure rickets in rats. llum, Simmonds, Becker and Shipley ('27) found that when ver oil was given to rats before mating, during pregnancy, and g the first two weeks of lactation, considerable protection against was afforded their young. As has already been noted, when ted yeast is fed to cows in sufficient amount, the vitamin D ned therein is passed on to a considerable extent by the cow,

so that milk containing as much as 400 I. U. per quart may be tained by this procedure. On the other hand, feeding cod liver oi liberal amounts to cows results in contributing little vitamin D their milk. Bunker, Harris and Eustis ('33) found that feeding 20 daily of mixed breast milk from women whose diets varied wid caused no healing of rickets in rats. On the other hand, a simi amount of breast milk from women who took daily 40 ounces milk, 24 ounces of which was from yeast-fed cows ("metabolize vitamin D milk) caused a definite antiricketic effect when fed fo days. This result indicates but a low antiricketic value in the milk number of other studies, which space does not permit to mention, in accord with these results.

Administration of Vitamin D during the Summer Season. The question whether administration of cod liver oil or other sou of vitamin D should be continued during the summer months often raised. Park points out that although theoretically this is n necessary, practically it is better to continue feeding vitamin D throug out the year. The sunlight in cities often contains little or no ultr violet rays, and is, therefore, not effective in preventing rickets. Als interruptions of feeding the vitamin often breaks down the habit taking it, on the part of both mother and child, and makes resumptio difficult or impossible. When there is a suspicion that cod liver o is causing digestive disturbances, or when it is distasteful, a concen trate or vitamin D milk should be used. Since the dose of vitamin D likely to prove insufficient when vitaminized milk is fed, the physicia should be consulted as to the source and dosage of the vitamin to b given, particularly in case of premature infants. It is sometimes neces sary to increase the dose even to 10,000 I. U. or more daily for suc infants in order to protect them. Park ('38) has discussed in detail th problems arising in vitamin D therapy in infants suffering fro various ailments.

Relation of the Distribution of Ultraviolet Light to the Geographic Distribution of Rickets.—Palm (1890, l.c.) was the first t express the view, based upon an extensive correspondence with medi cal missionaries in China, India, and elsewhere, that rickets is prin cipally caused by deficiency of sunlight, since its occurrence is com paratively rare where sunlight is abundant, and of frequent occurrenc where there is but little sunlight. Modern studies have brought t light the explanation of this relationship between climate and the dis tribution of the disease. In Northern countries, however, the paucity of sunshine during much of the year is compensated for by the character of the diet of the people, which consists largely of fish and fish-eating marine animals, bird skins, and at times, eggs. There are areas in the tropics where, because of excessive cloudiness, rickets

rs among children. Lack of sunlight, and of a dietary source of
nin D, also accounts for the prevalence of rickets in the infants
nothers who are secluded, as under Purdah, in India. It is in
erate regions, where lack of vitamin D in the food, together with
ity of sunshine during winter, and the wearing of heavy clothing,
iency of outdoor life, and in cities the absorption of ultraviolet
of the sunlight by the smokepall, that rickets has been most
alent. After the discovery of vitamin D in 1922, the tendency has
increasing throughout the world to provide vitamin D in some
for most infants and children. The result, viz., the reduction of
ncidence and severity of rickets in the young, has been one of the
est achievements in preventive medicine.

ir Pollution in Relation to Ultraviolet Light in Sunlight.—The
of solar ultraviolet due to a sooty atmosphere is of importance
a public health standpoint. The extent of this loss has been
ed out by a number of investigations measuring the intensity of
ght in rural and urban regions. Shrader, Coblenz and Korff ('29)
d that in the open country outside of Baltimore the amount of
violet light was about 50 per cent greater than in that city. A
y made by the U. S. Public Health Service ('34) of 14 of the
st cities in the United States, showed that there was 50 per cent
ultraviolet rays in the sunshine on clear days, in the least smoky
p, than in the most smoky group. On cloudy days there was 100
ent more. The maximum atmospheric pollution in all these cities
red during the winter months when the ultraviolet light is at a
mum because of the low altitude of the sun, cloudiness, smoke,
he shortness of the days. Even in clear weather, and in the coun-
unlight contains no appreciable ultraviolet light except when the
s above 30 degrees from the horizon. The extent to which air
tion may occur is shown by the study of Pincus and Stern ('37)
found that during a recent typical year, 300,000 tons of soot, tar,
rs, and ash were discharged into the air over New York City.
rtain areas of the city, soot and dust were found to settle at the
f 1800 tons per square mile per year. Figures as high or higher
been reported for other cities. Even in small cities, pollution may
bad when soft coal is burned as fuel in homes and factories.
statistics make it clear why the administration of some form of
in D is necessary for infants and children, and probably also
dults, throughout the year, except where sunshine is especially
lant.

sdall and Brown ('28) found the average antiricketic value of the
ine in Toronto during April to August, inclusive, to be about
times that during November to January, inclusive. They use the
"skyshine" to designate the sun's rays which are reflected from the

sky and clouds in contradistinction to the direct rays of the sun itself. They found the antiricketic value of skyshine to be approximately one-half to two-thirds that of direct sunlight. They suggest that rickets will not occur or will be very mild in regions where the minimum noonday altitude of the sun is 30 to 35 degrees, or less, for only a few weeks in the year. Places with a minimum altitude of 35 degrees lie along latitude 31° 30′, which runs a little south of Savannah, Ga. At the latitude of Glasgow, Scotland, (55°) the solar altitude is below 35° during six months of the year. In the latitude of Chicago the noonday altitude of the sun is below 35° for four months of the year. Clouds and fog cut off much of the ultraviolet light from the sun in certain places. P. A. Day ('32) states that the average daily amount of sunshine necessary to afford the same partial protection in rats receiving a rickets-producing diet, varied from 5 minutes in May, June, and July, to 168 minutes in December, with intermediate values for other months. Knudson ('32), using rats, found that in Albany, 90 to 120 minutes' daily exposure to sunshine during June to August inclusive, afforded complete protection, whereas exposures of 240 to 270 minutes daily during December and January caused little or no healing. Blunt and Cowan ('30) and Laurens ('33) have published extensive reviews on light in its relation to rickets.

General Metabolism.—Among the metabolic processes other than those involved in the prevention of rickets, vitamin D is concerned with energy metabolism.

A number of investigations have suggested a relation between antiricketic substances and the body's rate of gaseous exchange. Nitschke ('36) has pointed out that the basal metabolic rate may be considerably below normal in ricketic infants and rats, and that the rate is increased by vitamin D therapy. Administration of the vitamin to hedge-hogs prevented hibernation, a condition in which there is a depression of metabolism. Recently, Presnall ('37) demonstrated that vitamin D influences the rate of oxygen uptake by the skins of rats. The oxygen uptake of pieces of shaved skin from the back of young albino rats on an unsupplemented ricketogenic diet was only 35-84 per cent of that of skin from rats on the same diet but receiving 2.5 I.U. of vitamin D daily in the form of viosterol. When vitamin D was added to the diet of ricketic rats, the skin respiration increased as the rickets became healed. Further observations of a similar nature are those of Deutsch, Reed and Struck ('36), Siel ('29), and Landelius Ljungqvist ('34) who found that the lowered rate of oxygen consumption in rickets returns to normal with vitamin D intake. A finding of particular interest is that of Gelfan ('35), who demonstrated a response to vitamin D by a cold-blooded form of animal life when he showed that isolated muscles of frogs

ed with the antiricketic substance utilize more oxygen than do from normal frogs. Rappaport and collaborators ('33, '34) Reed and Dreyer and Reed ('35) have shown that massive doses itamin D considerably increase the metabolic rate of normal and rats.

he muscular weakness which occurs in rickets may be regarded ing the result of the disturbance in mineral balance. Since the l phosphorus is low, faulty muscle metabolism must be attributed pletion of body phosphorus. The phosphate ion plays a significant in carbohydrate metabolism and hexose phosphate is an active mediary in the cellular metabolism. This intimate metabolic iate of glucose and phosphate suggests that the depletion of phate in rickets may interfere with carbohydrate metabolism. ence that such is the case is indicated by Hentschel and Zöller who found that the inorganic and organic phosphate content e muscles was considerably reduced in rickets, and that in active ts the ability to synthesize hexosephosphate in the muscles is tly impaired. Landsberger and Silber ('27) found in florid rickets aired carbohydrate tolerance and a hyperglycemic curve that was counteracted by insulin.

More recently Sure and Smith ('31) reported no demonstrable ges in the concentration of blood sugar, alkaline reserve, or the ogen content of the liver in moderate or severe rickets. Freuden- and György ('20), Freudenberg and Welcker ('26), Adam ('27), Konig and Lenart ('27) observed a disturbance of the carbo- ate metabolism in rickets as indicated by an increase in the ase in the urine and feces and a decrease in the glycolytic power the lactic acid level of the blood serum. Konig and Lenart also d an increased alimentary glycemia in children with rickets. oni ('30) confirmed this observation.

aldwin, Nelson and McDonald ('28) believe that the antiricketic r is essential to normal carbohydrate metabolism in the chick. obtained changes in the respiratory quotient suggesting a rapid in ability to utilize carbohydrates. During the period that leg ness of the chicks was marked, the respiratory quotient indicated utilization of fats only, in spite of the fact that 47 per cent of diet consisted of carbohydrates. Administration of cod liver oil radiation brought the respiratory quotient to normal.

Hutchison ('20) noted no disturbance of the fat metabolism in ts. Telfer, however, ('26) reports that although the concentration t in the feces tends to be normal, combined fatty acids are vely lowered.

Liemann ('17) and Landsberger ('25) reported that ricketic ren are especially subject to ketosis. Hottinger ('28) found an

increase in the excretion of ketone acids in the urine. A hyp cholesteremia appears to be characteristic in rickets. The cholester concentration rises with the healing of rickets. (Dorlencourt ar Seiszoff, '29; Lesné, Sylvestre and Zizine, ('29). This however, ma be secondary to impaired carbohydrate metabolism.

Since the phosphate ion plays an important role in muscu metabolism not only through its association with glucose, but by i combination with creatine, it may be that loss of phosphate may als impair creatine metabolism. Further investigation on the effect o loss of this ion on fat metabolism might also yield significant result

Vitamin D depletion does not affect absorption of protein (S Julian and Heller, '30).

Vitamin D deficiency, apparently, does not directly affect hemato poietic function (Sure and Kik, '31). However, H. Day and Stein ('38) have noted that a deficiency of Vitamin D in diets containing an excess of phosphorus, relative or absolute, causes a greater degree of anemia than occurs with similar diets containing vitamin D.

Role of Vitamin D in Development of the Embryo.—From experiments with both animals and humans, evidence is available showing that a relation exists between vitamin D and embryonic development. Hess and coworkers ('28a), investigating the production of hens' eggs, observed that the concentration of vitamin D varies according to the diet and exposure to ultraviolet rays. Hens fed a vitamin D-deficient diet laid comparatively few eggs which contained little of the antiricketic factor. Fertile eggs from these hens failed to hatch. The hens' livers were also low in antiricketic potency. Experiments on young chicks tended to show that the lack of hatchability was due to lack of development of the embryo when the hens' diet was deficient in vitamin D, and was to be attributed to defective absorption of calcium from the shell into the yolk. Irradiated milk-fat preparations have been shown to increase the weight and hatchability of hens' eggs.

Hess and coworkers made similar studies on fish ('28b). They showed that the roe contained as much or even more of the antiricketic factor as the liver, whereas the newly hatched fish were practically devoid of it. They believe that this factor had been used up in the course of development of the larvae.

The number of studies on the effect of vitamin D on the development of the human embryo is small. Abels ('27) reported a case where cod liver oil administration during the second pregnancy resulted in the second child weighing 60 per cent more than the first. Similar results were observed by Vogt ('28).

Relation of Vitamin D to Linear Growth.—Stearns, Jeans and Vandecar ('36) and Slyker and associates ('37) have demonstrated

the rate of linear growth is influenced by the intake of vitamin D. erous amounts of vitamin D increase the skeletal growth. These kers present evidence that the greater rate of growth was not to any other factor, and the conclusion drawn from these experi- ts is that vitamin D is the chief factor affecting the rate of ar growth, since growth in length is dependent primarily on etal growth.

The Relative Effectiveness of Vitamins D_2 and D_3 from Dif- ent Sources for the Human Infant.—The question arises as to ther the human reaction to the different antiricketic substances is that of the rat or like that of the chicken. The evidence (Kramer Gittleman, '33; Drake et al., '34; Jeans and Stearns, '34; Wyman l., '35; Report of the Secretary of Agricultu e, '35) at present lable, while not conclusive, is at least indicative that, rat unit rat unit, the various forms of vitamin D have equal value for nans.

Hume ('37) has reviewed the literature relating to the relative ency of vitamin D from different sources for the rat, chick, and nan subject. She concludes that it "seems fairly certain that for human infant, vitamin D from fish liver oils, irradiated ergosterol, diated cholesterol, and vitamin D milks of various kinds, has the e relative potency as for rats." She further points out that sources itamin D for use with poultry should be standardized by means ests with chicks only, and the standard must be some source r than irradiated ergosterol. For practical purposes, any form of min D may safely be assayed by using the rat as a test animal. The importance of these observations at once attracted the attention ediatricians and stimulated much research. Park ('38, l.c.) lists rences to 39 such studies designed to show the comparative ncies of these different sources of vitamin D. He states: "Out he conflicting and most confusing results only generalizations of ntative nature are possible. For practical purposes calciferol, the min D in viosterol, may be regarded as being equal to the vitamin f cod liver oil. If viosterol is inferior to cod liver oil, rat unit for unit the difference cannot be great, or it would have come out e clearly." Dr. Park concludes from the data available that vitamin dispersed in milk, is more effective than in the usual oil menstrua. s may possibly be explained as due to its dissemination in the ute fat droplets, which could afford more effective absorption, erhaps on the assumption that it is advantageous to divide the ge as occurs in milk feedings. It is generally accepted that milk ched with vitamin D in the three ways mentioned, may, from practical point of view, be regarded as of equal value when inistered in quantities affording equal potencies as judged by the

rat assay method. He states that the evidence suggests that vitamin D in irradiated milk may be slightly superior to that in m of cows fed irradiated yeast, which is generally called "metaboli vitamin D." This term is misleading because there is no cha wrought by the metabolic activities of the cow upon the calcife administered to her in the form of irradiated yeast. The cow abso the calciferol and passes it on into her milk. The only appar superiority which such milk might possess over the other sources the vitamin would be due to better absorption and spacing of dosa due to milk feedings to the infant. In the absence of conclusi evidence to the contrary, the present indications are that the rat u is a satisfactory measure of the value of an antiricketic substance f humans, and that the question of source or form of vitamin D of significance only for chickens; the reaction of humans paralle that of rats and not that of chickens. The relative effect of differe sources of the vitamin on humans is directly proportional to th number of rat units which each provides.

Requirements for Vitamin D.—Vitamin D is needed throughou life, the requirement being greatest during infancy and during preg nancy and lactation. Rickets prevention alone can no longer b regarded as a criterion of adequacy of vitamin intake (Jeans, '36) The consensus of medical opinion is that feeding vitamin D i amounts well above the minimum levels necessary for prevention or cure of rickets has no deleterious effect, and that the rule should be to give it in ample quantity to cover any possible unrecognized requirement.

Since the periods of greatest susceptibility to rickets are the early months of life, it is important that vitamin D be given at the begin- ning of the third week, with a dose of one-half teaspoonful of cod liver oil, or of its equivalent in other preparations. This provides about 200 I.U. daily. After a few days, the dose should be increased to one teaspoonful of the oil, or a dose of 400 I.U. per day. During the fourth week, the infant should receive 800 I.U. daily, and if the oil is well tolerated, it may be increased to three teaspoonfuls during the second month. Twelve hundred to 1500 I.U. per day will do no harm. Eight hundred units per day should be given throughout the second year and thereafter. It should be pointed out that if any oil is allowed to run down the larynx into the lungs, it may cause pneumonia. Hence, the form in which the vitamin is given to sick infants should be determined by the physician, who will, in many cases, advise using a concentrate or vitamin D milk when vomiting occurs frequently and there is danger of the infant aspirating cod liver oil into the lungs.

For the prevention of rickets the regular administration of 400

. per day is regarded adequate. Jeans ('36, l.c.) and Jeans and arns ('38) have discussed the subject in detail, and have also pre-ted the views of the Committee on Foods of the American Medical sociation ('36). Park ('38, l.c.) suggests that for "working pur-ses," in the case of fish liver oils or viosterol, 800 to 1000 I.U. per y be regarded as the lowest levels which it is wise to give to the ant. Since irradiation of milk does not produce vitamin D in cess of about 135 I.U. per quart, such milk without a supplement the vitamin cannot be considered entirely satisfactory as a source of vitamin for infants or children.

There is a paucity of data on the vitamin D need of adults. In case of pregnant and lactating women, the League of Nations ealth Committee ('36) recommends a minimum daily intake of 0 I.U. However, there is reason to believe that the optimum for y age is several times greater than the minimal level necessary protection against hypovitaminosis D.

In rickets, the rapidity of cure depends on the dosage. The rapeutic dose of vitamin D has generally been regarded as 1000 to 00 I.U. per day for the human infant. Twelve hundred units, ovided by cod liver oil preparations, are considered adequate by nicians for rapid correction of rickets. When the vitamin is ad-nistered in the form of viosterol (calciferol) or irradiated ergosterol, should be given in larger doses than cod liver oil. A double unit se is recommended. The vitamin D of irradiated milk has given cellent clinical results with less than the usual dosage. Whether the ality of the vitamin, the vehicle, or extent of absorption of any ner factor is the reason for these differences is not as yet known.

Breast-feeding not a Specific Protective Factor Against Rickets. Hess ('29, l.c.) emphasized the fact that rickets has a lesser incidence ong nursing infants than among those fed cow's milk. He pointed t the tendency to credit breast milk with greater protective value an it actually possesses, and stated that "woman's milk is far from ng a specific." He cited observations by himself and Weinstock 7) as well as those of Macy and her associates ('28) which show t there is little, if any, vitamin D in certain samples of human lk. Macy and coworkers ('28) found that 25, 30, or 40 cc. daily human milk failed to cause healing of rickets in experimental rats. w's milk was effective when 30 cc. per day was fed to rats.

Coons and Coons ('32) found a much better retention of calcium ring pregnancy of women in Oklahoma than was noted by Coons d Blunt ('30) who studied women in Chicago. They attributed difference to the superior quality of the sunlight to which the uthern women were exposed. McBeath and McMahon ('33) studied o groups of breast-fed babies selected at the age of two months

from a pediatric clinic. The mothers of one of these groups we given daily one quart of vitamin D milk containing 432 I.U. p quart; while the mothers of the other group received a like amou of ordinary milk. Clinical and X-ray observations during six mont showed great differences in the two groups of babies. Of the babies whose mothers received vitamin D, 2 had severe rickets an 4 slight rickets, while 20 out of 26 in the control group had flor rickets requiring direct therapeutic treatment.

The Effects of Excessive Dosage of Vitamin D.—There are i the medical literature a number of statements to the effect tha irradiated ergosterol (calciferol) is an extremely toxic substance. Thi view arose through the use of an early German preparation, *vigantol* which was observed to cause pathological calcification in various part of the body. It is now clear that this preparation was toxic because (a) the ergosterol was irradiated in alcohol, which is not a suitable solvent in this connection, and (b) because the ergosterol was greatly over-irradiated, with the formation of toxic products. The present-day method of irradiating ergosterol dissolved in ether, with proper time regulation, avoids the formation of toxic products.

The margin of tolerance of the vitamin for man is not known. Numerous experiments suggest that there must be large individual variation. Excessive dosages of vitamin D have been given to adult patients as a treatment for chronic arthritis and psoriasis. Some of the patients apparently were able to tolerate such doses fairly well. An excellent review on this subject by Bills ('35) is available. When Wyatt, Hicks and Thompson ('36-37) administered 300,000 I.U. daily, they observed violent persistent nausea, intense headache, and profuse sweating. One patient suffered severe diarrhea, loss of appetite, and headache. These symptoms, together with nocturia and frequent micturation, have also been described by others. In over-dosage there are high concentrations of calcium and phosphate in the blood, and calcium casts have been observed in the urine, such as have been described by Albright and Bloomberg ('34) in cases of hyperparathyroidism. Elevation of the calcium content of the blood serum above 12 mg. per cent is an indicator of toxic effects of vitamin D.

Numerous investigators have demonstrated that the minimum toxic dose of vitamin D or of properly irradiated ergosterol is much greater than the therapeutic dose—as stated by Bills ('35, l.c.): "It is characteristic of vitamin D that the range between its ordinary therapeutic dose and the dose which produces adverse symptoms is extraordinarily wide; wider, perhaps, than is the case with any other potent drug."

Harris and Innes ('31) have reviewed the literature relating to hypervitaminosis D. Harris and Stewart ('29) showed that the ad-

istration of excess of vitamin D brings about hyperphosphatemia ypercalcemia, or both, in experimental animals. There is a high absorption of calcium and phosphate from the gut, and the severity ne hypervitaminosis D is regulated by the intake of these elements. ther words, the situation in hypervitaminosis D is the reverse of seen in deficiency of the vitamin.

A characteristic lesion in hypervitaminosis D is excessive calcifica- of the bone ending. The above mentioned investigators in their riments employed daily doses of irradiated ergosterol as high as g. per rat. An increase in the calcium content of the diet (or in Ca/P ratio) intensifies the hypervitaminosis and gives rise to eased formation of calcareous deposits in the kidneys, arteries, striated muscle, and other sites at a given level of vitamin D ss. When the diet was virtually devoid of calcium and phos- us, calcareous deposits were not produced by excess of vitamin but there was a greatly increased resorption of bone substance. ge doses of the vitamin stimulate osteogenesis, and in contrast rickets, a densely calcified overgrowth appears at the growing of the bone. Still greater excess causes extensive resorption, and cortex of the shaft and other compact bone becomes spongy. blood calcium and phosphate content are raised. It is not definitely blished to what extent increased absorption from the gut and eased withdrawal from the bones take place, but it is clear that min D in excess exerts a distributive action, resulting in deposition lcareous deposits at abnormal sites. Withdrawal of the vitamin ss causes greatly increased excretion of fecal calcium and phos- us. In some respects the mode of action of vitamin D appears e the reverse of that of the parathyroid hormone.

herapeutic Effects of Vitamin D Other than in Rickets.—The of vitamin D has been found beneficial in diseases other than ts. Some of these will be presented here.

nfantile Tetany: Tetany is associated with a hypocalcemia as rasted with the hypophosphetemia of rickets (Miraglia, '26; rgy, '29). Ultraviolet rays are capable of raising the calcium of the blood to normal. Of the group of substances which exert rative effect on tetany, irradiated ergosterol is the best (Gleich Goodman, '28; Rohmer et al., '28; Bakwin et al., '29; Wilkes, '29). ing and Hopper ('36) found that tetany did not develop in any eir cases receiving vitamin D.

steomalacia: Osteomalacia represents the manifestations of ts as they appear in the adult, where the element of growth is nt, resorptive processes are in the ascendancy and bone softening deformity are the outstanding features of the disease. This se affects mainly pregnant and lactating women. It occurs in

many parts of the world, notably in India, China and Japan. Numerous workers have pointed out the close relation between vitamin D and osteomalacia. Pommer (1885), in his studies upon the histological changes in osteomalacia and rickets, concluded that the essential abnormality in each disease was a cessation of calcification. Schmorl ('06) also found evidence of deficiency of provisional calcification and irregularity of endochondral ossification in young subjects of osteomalacia, changes similar to the disturbances of endochondral ossification in rickets. Hess ('29, l.c.) believed that the differences in the pathological lesions in rickets are quantitative rather than qualitative. The underlying pathological process is the same. Maxwell ('30) claimed that true osteomalacia is the manifestation of rickets in bones that have reached maturity and is connected with a shortage of vitamin D. When a source of vitamin D is provided, and the diet is improved in quality generally, striking improvement in cases of osteomalacia takes place. (Maxwell, '30, l.c.; Wilson and Surie, '30; Hottinger, '27; Lasch, '28; Green-Armytage, '28; Mathez, '29).

Celiac Disease: This disease is often followed by development of rickets, osteoporosis, and tetany. One marked feature of this condition is the loss of large amounts of undigested fat which is excreted in the feces. Since vitamin D is associated with dietary fats, it is believed that absorption of this nutrient is faulty in celiac disease even though the quality and quantity of the diet is adequate for protection against rickets. It is also thought that absorption of calcium is impaired due to the formation of calcium soaps. Dietotherapy is used in these cases and the omission from the diet of fat-containing foods is customary. Vitamin D is administered by mouth or frequently the individual is irradiated. Neale, Smallwood and Shippam ('36) and Shelling and Hopper ('36, l.c.) reported recovery and normal development of children with celiac disease upon treatment with vitamin D.

Treatment of Arthritis with Massive Doses of Vitamin D: Rappaport, Reed and associates ('33 l.c., '34 l.c., '35 l.c.) reported abatement of the symptoms of arthritis (atrophic, both rheumatoid and infectious; hypertrophic, both degenerative and menopausal, and mixed cases) when 200,000 I.U. per day of vitamin D were administered as viosterol, the signs of improvement appearing after periods of 1 week to 6 months. In some cases, they increased the dosage to 1,000,000 I.U. per day. There was a fairly high percentage of failures. Livingston ('36), Wyatt, Hicks and Thompson ('36, l.c.), and Farley ('37) have also used this therapy successfully. The mechanism of the clinical improvement, when it occurs, is not yet understood.

Treatment of Hay Fever with Massive Doses of Vitamin D: paport and Reed and coworkers ('33, l.c., '34 l.c.) studied the effect viosterol on allergic conditions. The viosterol employed was ially prepared, about 100 times stronger than commercial 250 D. ntained 920,000 U.S.P. (I.U.) units per cc. This treatment gave lerate to complete relief to 82.4 per cent of the cases. Analysis ved that the relief was due not to an increase in the total calcium o any consistent alteration in the calcium partition of the blood. vever, the K-Ca ratio became lower and less fluctuating.

Treatment of Psoriasis with Vitamin D: Psoriasis is a disorder he skin common among peoples in northern regions. It is more re in the winter than in summer.

While studying the effect of large doses of vitamin D in arthritis, er and Zon ('37) observed complete disappearance of the psoriasis patient who had both arthritis and psoriasis. Vitamin D was administered to a group of individuals who had chronic, wide-ad psoriasis of several years' duration. No local or dietary treat-t was employed. Direct sunlight was excluded as much as possible ng the summer months. After 6 to 12 weeks of vitamin D apy the disease was checked. There was recurrence in some cases, it was gradual and less severe. These investigators suggest, there-, that vitamin D is effective in the treatment of psoriasis.

Trichinosis and Vitamin D Therapy: The possible relationship ween trichinosis and vitamin D therapy has been investigated. In disease when the protective mechanism begins to function, cysts in to form about the larvae in the muscular tissues. These cysts ome calcified and as the calcification progresses the disease abates. an attempt to determine whether or not this protective calcification ght be hastened by the administration of vitamin D, Wantland) conducted an experiment on rabbits suffering with trichinosis. cium lactate and varying amounts of irradiated ergosterol were inistered. The rate of calcification of the cysts was found to vary ctly with the amount of vitamin D and calcium lactate given. rked calcification of the cysts was obtained after four to six weeks. ntrol animals showed no calcification of cysts after periods of from eeks to 3 months. Whether or not similar results would follow min D therapy in cases of human trichinosis has not yet been nonstrated.

Eye Changes in Vitamin D Deficiency: Blackberg and Knapp) state that in pups restricted to a diet deficient in vitamin D low in calcium, there was a weakening of the cornea and sclera, ch they regard as apparently identical with changes in the eyes man, the cause of which was unknown. Since these changes are cerned with the refractive properties of the eye, they suggest that

human myopia may be dependent in part upon a deficier calcium, phosphorus, and vitamin D. They have also reported favorable results in such cases by rectifying these dietary defici

Effect of Vitamin D on Coagulation Time: In surgica cedures of gall bladder disorders, slow clotting of the blood serious problem. McNealy, Shapiro and Melnick ('35) reporte operative administration of viosterol, with bile salts to aid absorption, to be an effective means of shortening the time of co tion of the blood. Johnston ('37) also has employed this m with success. In three patients before treatment the coagulation was 480, 275, and 480 seconds, respectively. The normal time is 240 seconds. After treatment the times were reduced to 180, 225 240 seconds respectively. He also administered glucose and ca intravenously. Such results as are available indicate that the ca level of the blood is not generally reduced in this condition.

Vitamin D and Calcium Therapy in Pregnancy Toxe Theobald ('37) states that the daily administration of vitamin and D, and calcium, reduced the occurrence of albuminuria, vomi high blood pressure, headaches, edema, cramps, and insomni pregnant women. He observed 100 healthy women, not more 24 weeks pregnant, who were allocated in two groups at rand One group was directed to take daily 20 gm. of calcium lac 11,000 I.U. of vitamin A, and 450 I.U. of vitamin D. The other gr did not take any supplements. All were observed until the termina of pregnancy. The above named symptoms occurred 23 times am the women receiving the supplements, whereas in the non-sup mented group they occurred 49 times. In the treated group, 8 veloped albuminuria and/or hypertension, and 6 had other sympt of toxemia. In the untreated group the corresponding figures w 17 and 11. He regards the results as statistically significant in fa of the nutritional therapy. This view is supported by the fact those of the cases which were admitted to the hospital after deve ment of symptoms, lost their symptoms on dietetic treatment. O ously, further observations will be necessary to establish the valu this treatment.

Milk Fever: Milk fever is generally believed to be caused deficiency of calcium in the blood. Since the subject is discusse Chapter VII, it will suffice to note here that the evidence avail supports the view that administration of vitamin D before the yo are born will tend to prevent the disease.

Vitamin D in Resistance to Infantile Paralysis: Since the q tion of the portal of entry of the virus of poliomyelitis is still be strongly debated, no appraisal is attempted here of the evidence u which divergent views of investigators rest. It is appropriate, howe

call attention to the view of Toomey ('37) which he supports weighty evidence, that infection with the virus may be by way the gastrointestinal tract. He found normal monkeys difficult to ct by means of this path, but monkeys suffering from rickets easily infected by this route. He expresses the belief that the ead of the virus is usually stopped in normal animals by some or in the white and not in the grey fibers. The former contain per cent of cholesterol, the latter but 0.7 per cent. Cholesterol orbs and destroys the poliomyelitis virus. When rickets is produced rats by vitamin D deficiency, the nerve myelin becomes involved, racterized by a foamy appearance attributed to edema of the rokeratin framework. When rickets is produced in monkeys, it ssumed that the nerve myelin and its cholesterol become involved; virus is not absorbed or destroyed, thus accounting for the ease infection of such monkeys. On the other hand, when monkeys e had plenty of vitamin D, and inferentially normal myelin sheaths, disease cannot be produced *via* the gastrointestinal tract.

It should be pointed out that vitamin D increases the permeability the intestinal mucosa to calcium and phosphate (or decreases retion). It is conceivable that altered permeability, in the opposite se, to the virus may occur.

Role of Vitamin D in Wound Healing: Bond ('28), as well as ers, has described the clinical application of vitamin D as cod r oil or irradiated ergosterol. Bond observed that irradiation of osterol produced a fat-soluble substance containing oxygen. He noted that ozonization of an ergosterol film converted ergosterol an oxidizing substance, probably a peroxide or ozonide of osterol. Whether this substance is identical with vitamin D, or ether the formation of the peroxide is a subsidiary reaction during process of vitamin D formation is not clear, but Bond suggests the action of irradiated ergosterol on wounds and granulating faces may be due to the peroxide rather than to the vitamin.

Fractures and Vitamin D: Vitamin D has been observed to rt a healing effect in the delayed or imperfect union of fractures old people and children and in all conditions which are accom- ied by decalcification of the epiphyses (Bors, '27; Roi, '30; Collazo l., '30; Knoflach, '28; Morelle, '29; Roederer, '29; Cuthbertson, '30).

Vitamin D in Relation to Dental Caries.—A separate chapter levoted to the relation of the diet to tooth development and to h preservation, therefore only a few observations will be recorded which bear upon this subject.

Since both the enamel and dentin of teeth consist of mineral osits made up principally of calcium phosphate, with some onate, their formation is obviously a biological process involving

a calcifying mechanism. Any calcifying process involves cellular tivity, and a proper nutrient medium in which the cells may funct The state of nutrition, as respects those factors which influence ei of these tissues will influence, for good or bad, the structure of teeth.

The organ which applies the enamel to the developing tooth the ameloblast. Each cell builds a single enamel rod or prism calcified substance. These are fitted together as a mosaic to form tooth enamel. The ameloblasts are specialized cells derived fr epithelial tissue, and in accord with the special sensitivity of epithe tissues generally, to deficiency of vitamin A, it is shown t ameloblasts share this sensitivity. Hence if the diet is optimum other respects, but deficient in vitamin A, the ameloblasts will abnormal histologically and functionally, and defective enamel w be formed. If the diet is deficient in ascorbic acid, the odontobla will be unable to lay down normal dentin. If both vitamins A and are provided in abundance, but the calcium and phosphate conte of the blood is too low, as occurs in deficiency of vitamin D, and a lesser extent in deficiencies of calcium or phosphorus, or both, bo the ameloblasts and odontoblasts will be unable to form their norm products, enamel and dentin.

Due to the fact that in temperate regions almost all foodstu are deficient or lacking in vitamin D, and until the past fifteen yea adequate supplements of this vitamin were generally not provide to infants, children, and pregnant and lactating women, and th prevalence of rickets was widespread, it is evident that deficiency vitamin D must have had an unfavorable effect upon the structu of the teeth. Because defective enamel predisposes teeth to deca this deficiency must have contributed to the high incidence of dent caries of persons who passed through infancy in the pre-vitamin days.

Since sunlight containing adequate amounts of ultraviolet ray acting upon the skin, is a source of vitamin D, dental caries woul be expected to occur more frequently in children in the northe states than farther south. Of interest in this connection is a surve begun in 1933 under the auspices of the American Dental Associatio in which 8,000 dentists in 26 states collaborated, and in which ov 1,400,000 children were examined, representing 11.6 per cent of th estimated number of children 6 to 14 years of age in the areas studie The results were published in 1936 by the U. S. Public Health Servic In the Southern States 60 per cent of the children had caries of th permanent teeth, whereas in the Northern States the percentag was 76. In the South the number of cavities per 100 children wa 202; in the Northern States the figure was 293. In searching for th

se or causes of this difference in incidence of dental caries, one y postulate that the diet, in one or more respects, is inferior in Northern States to that in the South. In the absence of more de- ed data this factor cannot be evaluated. It seems logical to attribute difference in incidence of tooth decay, in some measure at least, deficiency of effective sunshine in the North. In 12 Northern cities etween latitudes 37° and 43°) in February at 9 a. m., the ultra- let light was only about one-ninth as intense as it was in a similar mber of Southern cities (between latitudes 29° and 35°). For the nth of November at the same hour the intensity of ultraviolet nt was about one-fourth as great in the Northern cities. At on the Northern cities received about one-half as much ultraviolet nt. The six states showing the highest incidence of dental caries re Maine (3.97 per child), Pennsylvania (3.74), Massachusetts 65), Wisconsin (3.63), Ohio (3.31), and New Jersey (3.02).

M. Mellanby ('29-'36), Boyd, Drain and Stearns ('37) and others ve reported partial control of dental caries by the provision of an equate diet, and have clearly achieved the arrest of caries in some es, through the administration of vitamin D. The role of this tor will be further considered in the chapter on the diet in relation teeth.

REFERENCES

MMER (1885). "Untersuchungen über Osteomalacie und Rachitis." Leip- ig.

M (1890). "The Practitioner." *45*, 270, 321.

CHHOLZ (1904). Verhandl. d. Gesselsch. f. Kinderheilk., *21*, 216.

LDSCHINSKY (1919). Deutsche med. Wchnschr., *45*, 712.

LDSCHINSKY (1929). Zeit. f. orthop. Chir., *39*, 426.

s and UNGER (1920-21). Proc. Soc. Exp. Biol. & Med., *18*, 298.

LLANBY (1929). Lancet, *i*, 407.

LLANBY (1921). Med. Res. Council, Spec. Rep. Ser., No. 61, London.

KINS (1920). Biochem. J., *14*, 725.

OLLUM, SIMMONDS, PARSONS, SHIPLEY and PARK (1921). J. Biol. Chem., 5, 333.

OLLUM, SIMMONDS, BECKER and SHIPLEY (1922). J. Biol. Chem., *53*, 3.

MAN and PAPPENHEIMER (1921). Proc. Soc. Exp. Biol. & Med., *18*, 193; Exper. Med., *34*, 189.

ENHEIMER, MCCANN, ZUCKER and HESS (1921-22). Proc. Soc. Exp. ol. & Med., *18*, 276.

IL, BROWN, CHAPMAN, ROSE and SAUERWEIN (1932). J. Biol. Chem., *98*, 7.

. (1923). Physiol. Rev., *3*, 106.

Hess (1929). "Rickets, Including Osteomalacia and Tetany." Lea Febiger, Philadelphia.
Howland and Kramer (1921). Am. J. Dis. Child., *22*, 105.
Kramer, Tisdall and Howland (1921). Am. J. Dis. Child. *22*, 431.
Kramer and Howland (1922). Bull. Johns Hopkins Hosp., *33*, 313.
Brown, Tisdall, Courtney and MacLachlan (1922). Arch. Pediat., 559.
Howland and Kramer (1922). Trans. Am. Ped. Soc., *34*, 204.
Howland and Kramer (1923). Monatschr. f. Kinderheilk., *25*, 279.
Hess, Weinstock, Rivkin and Gross (1930). J. Biol. Chem., *87*, 37.
McGowan (1933). Biochem. J., *27*, 934.
Kramer and Howland (1932). J. Nutr., *5*, 39.
Orr, Holt, Wilkins and Boone (1923). Am. J. Dis. Child., *26*, 362.
Stolzberg and Meyer (1932). Monatschr. f. Kinderh., *54*, 195.
Rominger, Meyer and Bomskov (1932). Monatschr. f. Kinderh, *55*,
Kay and Guyatt (1933). Nature, *131*, 468.
Shohl and Bennett (1927). J. Biol. Chem., *74*, 247.
Shohl and Bennett (1928). J. Biol. Chem. *76*, 633; *78*, 181.
Karelitz and Shohl (1927). J. Biol. Chem., *73*, 655, 665.
Williams (1928). Am. J. Dis. Child., *35*, 590.
Warkany (1930). Ztschr. f. Kinderh., *49*, 191.
Shipley, Kramer and Howland (1926). Biochem. J., *20*, 379.
Murdoch (1927). Arch. Dis. Child., *2*, 285.
Courtney, Tisdall and Brown (1928). Canad. Med. Assoc. J., *19*, 559
Koch and Cahan (1927). Am. J. Dis. Child., *34*, 187.
Daniels, Stearns and Hutton (1929). Am. J. Dis. Child., *37*, 296.
Skaar (1927). Norsk. Mag. f. Laegevidensk., *88*, 665.
Skaar and Haupl (1929). Virchow's Arch. f. path. Anat., *271*, 100.
Wilder and Howell (1936). J. Am. Med. Assoc., *106*, 427.
Hamilton and Schwartz (1932). J. Clin Investig., *11*, 817.
Eckstein and Wile (1926). J. Biol. Chem., *69*, 181.
Hess and Weinstock (1925). J. Biol. Chem., *64*, 18.
Rekling (1927). Strahlentherapie, *25*, 568.
Hou and Tso (1928). Chinese J. Physiol., *2*, 345.
Hou and Tso (1929). Chinese J. Physiol., *3*, 171.
Hou and Tso (1930). Chinese J. Physiol., *4*, 79, 345.
Hou (1930). Chinese J. Physiol., *4*, 93.
Rowan (1928). Nature, *121*, 323.
Hou (1931). Chinese J. Physiol., *5*, 79, 345.
Helmer and Jansen (1937). Studies of the Institutum Divi Thomas, 207.
Hume, Lucas and Smith (1927). Biochem. J., *21*, 362.
Astrowe and Morgan (1935). Am. J. Dis. Child., *49*, 912.
Amrhein (1934). Am. Pharm. Assoc., *23*, 182.
Hess, Weinstock and Hellman (1925). J. Biol. Chem., *63*, 305.
Fodor (1934). Ztschr. f. Vitaminforsch., *3*, 241
Hess and Weinstock (1924a). J. Am. Med. Assoc., *83*, 1558.
Toverud and Toverud (1931). Acta Paed., *12*, Suppl. II.

MANN (1937). J. Biol. Chem., *118*, 371.
STENBERGER (1933). Monatschr. f. Kinderh., *56*, 217.
AVES and SCHMIDT (1933). J. Biol. Chem., *102*, 191.
MANN (1937a). Proc. Soc. Exp. Biol. & Med., *36*, 812.
MANN (1937b). J. Biol. Chem., *122*, 249.
MANN (1937c). J. Biol. Chem., *122*, 257.
S and WEINSTOCK (1924b). J. Biol. Chem., *62*, 301.
STENBERGER, HARTMAN and SMITH (1927). California & West. Med., *27*, 0.
OLLUM, SIMMONDS, BECKER and SHIPLEY (1927). Am. J. Dis. Child., 3, 230.
KER, HARRIS and EUSTIS (1933). New England J. Med., *208*, 313.
S (1938). J. Am. Med. Assoc., *111*, 1179.
ADLER, COBLENZ and KOREF (1929). Am. J. Pub. Health., *19*, 717.
S. Public Health Service (1934). U. S. Treas. Dept. Pub. Health Bull., O. 224.
CUS and STERN (1937). Am. J. Pub. Health., *27*, 321.
ALL and BROWN (1928). Trans. Am. Ped. Soc., *40*, 50.
(1932). Am. J. Dis. Child., *43*, 1455.
DSON (1932). Proc. Soc. Exp. Biol. & Med., *30*, 66.
NT and COWAN (1930). "Ultraviolet Light and Vitamin D in Nutrin." University of Chicago Press, Chicago.
RENS (1933). "The Physiological Effects of Radiant Energy." Chemical talog Co., New York.
CHKE (1936). Deutsche med. Wchnschr., *62*, 629.
NALL (1937). J. Biol. Chem., *121*, 5.
TSCH, REED and STRUCK (1936). Am. J. Physiol., *117*, 1.
(1929). Arch. f. exper. Path. u. Pharmakol., *140*, 194.
ELIUS LJUNGQVIST (1934). Skandinav. Arch. f. Physiol., *68*, 252.
AN (1935). Am. J. Physiol., *113*, 464.
APORT and REED (1933). J. Am. Med. Assoc., *101*, 105.
APORT, REED, HATHAWAY and STRUCK. (1934). J. Allergy, *5*, 541.
(1934). Arch. Phys. Therap., X-ray, Rad., *15*, 69.
ER and REED (1935). Arch. Phys. Ther., X-ray, Rad., *16*, 537.
TSCHEL and ZÖLLER (1926). Klin. Wchnschr., *5*, 2283.
SBERGER and SILBER (1927). Jahrb. f. Kinderh., *117*, 9.
and SMITH (1931). Proc. Soc. Exp. Biol. & Med., *28*, 440.
DENBERG and GYÖRGY (1920). München. med. Wchnschr., *37*, 1061.
DENBERG and WELCKER (1926). Ztschr. f. Kinderh., *41*, 466.
(1927). Klind. Wchnschr., *6*, 1289.
and LENART (1927). Jahrb. f. Kinderh., *115*, 271.
ONI (1930). Arch. de méd. d. Enf., Paris., *33*, 129.
VIN, NELSON and MCDONALD (1928). Am. J. Physiol., *85*, 482.
HISON (1920). Quart. J. Med., *13*, 277.
R (1926). Quart. J. Med., *20*, 7.
ANN (1917). Jahrb. f. Kinderh., *85*, 210.
BERGER (1925). Ztschr. f. Kinderh., *39*, 597.
NGER (1928). Naturwissenschaften., *16*, 484.

Dorlencourt and Seiszoff (1929). Bull. Soc. Pediat., *27*, 352.
Lesné, Sylvestre and Zizine (1929). Bull. Soc. Pediat., *27*, 350.
St. Julian and Heller (1930). J. Biol. Chem., *90*, 99.
Sure and Kik (1931). Proc. Soc. Expt. Biol. & Med., *28*, 496.
Day and Stein (1938). J. Nutr. 16, 525.
Hess, Bills, Weinstock, Honeywell and Rivkin (1928a). Proc. S
Exp. Biol. & Med., *25*, 651.
Hess, Bills, Weinstock, Honeywell and Rivkin (1928b). Proc. S
Exp. Biol. & Med., *25*, 652.
Abels (1927). Wien. klin. Wchnschr., *40*, 1105.
Vogt (1928). München. med. Wchnschr., *53*, 1634.
Stearns, Jeans and Vandecar (1936). J. Pediat., *9*, 1.
Slyker, Hamil, Poole, Cooley and Macy (1937). Proc. Soc. Exp. Biol
Med., *37*, 499.
Kramer and Gittleman (1933). New England J. Med., *209*, 906.
Drake, Tisdall and Brown (1934). Canad. Med. Assoc. J., *31*, 1159.
Jeans and Stearns (1934). Proc. Soc. Exp. Biol. Med., *31*, 1159.
Wyman, Eley, Bunker and Harris (1935). New England J. Med., 2
257.
Report of the Secretary of Agriculture (1935). U. S. Printing Offi
p. 103.
Hume (1937). Nutr. Abst. & Rev., *6*, 891.
Jeans (1936). J. Am. Med. Assoc., *106*, 2066, 2150.
Jeans and Stearns (1938). J. Am. Med. Assoc., *111*, 703.
Committee on Foods (1936). J. Am. Med. Assoc., *106*, 1734.
Nutrition Committee, League of Nations (1936).
Hess and Weinstock (1927). Am. J. Dis. Child., *34*, 845.
Macy, Outhouse and Brekke (1928). J. Biol. Chem., *78*, 129.
Coons and Coons (1932). Rep. Div. of Biol. Chem., Am. Chem. Soc., De
ver, Col., Aug.
Coons and Blunt (1930). J. Biol. Chem., *86*, 1.
McBeath and McMahon (1933). Wis. Med. J., *32*, 385.
Bills (1935). Physiol. Rev., *15*, 1.
Wyatt, Hicks and Thompson (1936-37). Ann. Internal Med., *10*, 534.
Albright and Bloomberg (1934). Trans. Amer. A. Genito-Urin. Surgeon
27, 195.
Harris and Innes (1931). Biochem. J., *25*, 367.
Harris and Stewart (1929). Biochem. J., *23*, 206.
Miraglia (1926). La Pediatria., *34*, 1116.
György (1929). "Die Behandlung und Verhutung der Rachitis und T
tanie." Julius Springer, Berlin.
Gleich and Goodman (1928). New York State J. Med., *28*, 1398.
Rohmer, Woringet and Anderson (1928). Bull. Soc. de Pediat. de Pari
26, 128.
Bakwin, Bakwin and Gottschall (1929). Am. J. Dis. Child., *37*, 31
Wilkes (1929). Am. J. Dis. Child., *37*, 483.
Shelling and Hopper (1936). Bull. Johns Hopkins Hosp., *58*, 137.
Schmorl (1906). Deutsche. Arch. f. Klin. Med., *86*, 170.

WELL (1930). Lancet, *1*, 454; Proc. Roy. Soc. Med., *23*, 639.
SON and SURIE (1930). Indian J. Med. Res., *30*, 889, 903.
TINGER (1927). Ztschr. f. Kinderh., *44*, 282.
CH (1928). Klin. Wchnschr., *7*, 2148.
EN-ARMYTAGE (1928). Ind. Med. Gaz., *63*, 357.
HEZ (1929). Rev. Med. de la Suisse Romande, *49*, 158.
LE, SMALLWOOD and SHIPPAM (1936). Am. J. Dis. Child., *50*, 1502.
NGSTON (1936). Arch. Phys. Ther., X-ray, Rad., *17*, 702.
LEY (1937). Ill. Med. J., *71*, 74.
ER and ZON (1937). U. S. Pub. Health Reports, *52*, 1580.
NTLAND (1934). Proc. Soc. Exp. Biol. & Med., *32*, 438.
CKBERG and KNAPP (1934). Arch. Ophth., *11*, 665.
CKBERG and KNAPP (1937). Am. J. Ophth., *20*, 405.
NEALY, SHAPIRO and MELNICK (1935). Surg. Gynec. and Obstet., *60*, 5.
STON (1937). J. Med., *18*, 235.
OBALD (1937). Lancet, *232*, 1397.
MEY (1937). J. Am. Med. Assoc., *109*, 402.
D (1928). Brit. Med. J., *1*, 339, 642.
(1927). Zentralbl. f. Chir., *54*, 3266.
(1930). Reforma Medica Naples, *45*, 1551.
LAZO, RUBINO and VARELA (1930). Archiv. f. klin. Chir., *158*, 214.
FLACH (1928). Wien. Klin. Wchnschr., *41*, 739.
ELLE (1930). Rev. Belge du Science Med., *2*, 226.
DERER (1929). Bull. et Mém. Soc. de Méd. de Paris, *3*, 72.
HBERTSON (1930). Biochem. J., *24*, 1244.
. Public Health Service (1936-37). U. S. Treas. Dept. Pub. Health rvice Bull. Nos. 226 and 233.
LANBY (1929-1936). Med. Res. Council, Special Rep. Ser. No. 140, 3, 191, 211.
, DRAIN and STEARNS (1937). Proc. Soc. Exper. Biol. & Med., *36*, 645.

CHAPTER

XVI

Chemical Nature of Ascorbic Acid (Vitamin C)

IN NO DEPARTMENT of nutritional research has there bee dramatic progress than in that relating to the nature of scur its prevention through the isolation and characterization of the factor, ascorbic acid, the deficiency of which causes scurvy. I formity with the vitamin hypothesis there was for years a pre belief that scurvy had its origin in the deficiency of a specific factor. The isolation of this substance eluded workers for mor a decade and after the task had been accomplished by Szent-G in 1927 it was not until five years later that anyone, inc Szent-Györgyi, knew that it was vitamin C. The chemical methods of determination, and distribution of this interestin stance will constitute the principal content of this chapter.

Nomenclature.—There has been some confusion regardi nomenclature of ascorbic acid. Originally the factor which pr scurvy was designated as the *antiscorbutic vitamin*. Gradual was more or less replaced by the term *vitamin C* during the part of the nineteen twenties. In 1933 Szent-Györgyi and Ha named the substance *ascorbic acid*. This designation met with g approval among chemists, both here and abroad, but owing therapeutic suggestiveness the American Medical Association a name without that objectionable connotation. The Coun Pharmacy and Chemistry of the American Medical Asso adopted a new designation, *cevitamic acid,* and attempted to g general acceptance but the effort failed. Recently the Council doned the new name. For the sake of simplification in v designation it is probable that the term, *ascorbic acid,* will gra supersede all others, since it has been generally employed by ch from the time it was originated in 1933.

Isolation and Identification of Ascorbic Acid.—King ('36) has iefly reviewed the major facts concerning this subject and, as pointed t in his review, the principal workers were Zilva et al. (Zilva, '28), ezssonoff and associates (Bezssonoff, '31), and King and coworkers mith and King, '31). Also, Cox ('37) has outlined some of the portant contributions in the isolation and identification of this trient. In connection with his studies of reducing substances in rious tissues, Szent-Györgyi ('27) isolated a compound which . . . was named by its protocol number 'C_{xii}' being the twelfth bstance prepared and examined in my work on tissue oxidation d the function of the adrenal cortex." The use of the letter "C" as fortuitous and without implication that the substance was related vitamin C. Since it appeared five years later that this compound as vitamin C it is of interest to discuss briefly some facts regarding behavior in animal and plant tissues.

Food chemists had for many years relied upon the guaiacum action as a test to determine whether a substance, *e.g.* milk, or uit juice, had been heated. If to a small amount of a solution of m guaiac in water a few drops of hydrogen peroxide is added, the aiaconic acid present is not oxidized. If now a few drops of raw tato juice, or other unheated vegetable juice, or raw milk is added, e hydrogen peroxide is decomposed, with the formation of active ygen, which oxidizes the guaiaconic acid, and the solution turns ue-green. The enzyme decomposing the hydrogen peroxide is called roxidase. If the juice has been heated above 78° C. the peroxidase destroyed. Szent-Györgyi was the first to possess the acumen to serve that when this reaction is carried out using a solution of rified peroxidase plus a few drops of an alcoholic solution of gum aiac and a few drops of 0.01 N hydrogen peroxide, the development the blue-green color is immediate. When the same reagents are plied to the press juice of a plant which contains peroxidase, the ction remains negative and no color appears. If, however, the antity of hydrogen peroxide is gradually increased, after a certain ount has been added, the color suddenly appears and grows deeper h the further addition of peroxide. He demonstrated that the ct of natural juices was not due to inhibiting substances, as had n supposed, but to the consumption of the first active oxygen made ilable by another reaction. He was able to separate in crystalline m the substance which, in this reaction, takes precedence to the iaconic acid in using up active oxygen, the latter becoming dized only after the former has been used up in the oxidation cess. The new substance had the empirical composition $C_6H_8O_6$. ce it was isomeric with the lactone of glycuronic acid it was eved to belong to the uronic acid group and was referred to as

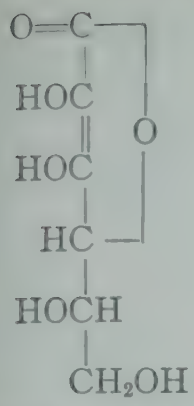
hexuronic acid (Szent-Györgyi, '28). At that time, 1928, Szent-Gyö believed the substance to be associated with vitamin C but tha was not vitamin C itself. This view was shared by Zilva ('32).

In this country King and associates (McKinnis and King, Smith and King, '31, l. c.) were actively engaged in investigation relationships between vitamin C and "hexuronic acid." Finally April 1, 1932, King and Waugh ('32) announced the isolation vitamin C in crystalline form and stated that, "The recrystalli substance corresponds in chemical and physical properties to a h uronic acid, and is apparently identical with the hexuronic a described by Szent-Györgyi. . . ." On April 16, 1932, Svirbely (w had been recently associated with King in work on vitamin C c centration) and Szent-Györgyi ('32a) announced that they had, means of 1 mg. of hexuronic acid daily, protected guinea pigs fr scurvy during 56 days. But it was not until May 7, 1932, that th (id. '32b) claimed definitely the identity of hexuronic acid w vitamin C. These fundamental studies of Szent-Györgyi and of Ki et al. have been amply confirmed by other workers. It is of p ticular interest that Tillmans and associates ('32) pointed out t marked correlation between the antiscorbutic value of foods a their quantitative reduction of 2,6-dichlorophenolindophenol, t oxidation-reduction indicator widely used at present in the chemi determination of ascorbic acid.

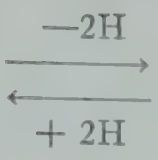
Following identification of the important material, isolated ind pendently by King and associates and Szent-Györgyi, attention w focused on the improvement of methods of concentration. Waug Bessey and King ('33) were able to secure 100 to 150 mg. of ascorb acid per liter of lemon juice. Szent-Györgyi's discovery of papri as a convenient source of the natural vitamin made it possible for hi to prepare several hundred grams for the international cooperati study of its chemical nature.

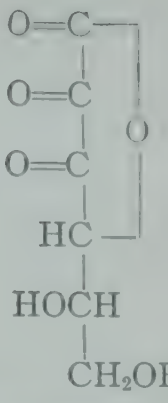
Structural Formula of Ascorbic Acid.—The structural formu of ascorbic acid was determined by a series of brilliant investigatio by Hirst, Haworth, and their coworkers (Cox, Hirst and Reynol '32), Karrer and associates (Karrer, Salomon, Morf and Schöpp, '3 Karrer, Schwarzenbach and Schöpp, '33; Karrer, Schöpp a Zehender, '33), von Euler and Klussmann ('33), and Micheel a Kraft ('33). Other investigators also contributed to this importa work.

Among several formulae proposed, the following, along with i reversibly oxidized form, is now generally accepted as best accountin for all of the vitamin's properties:



-ascorbic acid (vitamin C)

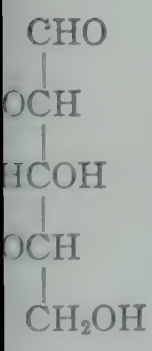




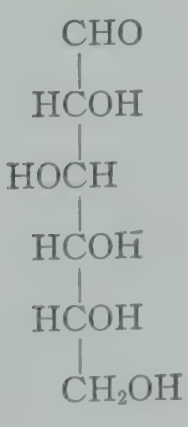
dehydroascorbic acid

Important evidence concerning the molecular structure was contributed by Herbert, Hirst, Percival, Reynolds and Smith ('33), who studied the behavior with oxidizing agents. The data indicated that the compound is related to l-gulose. The first oxidation product was 3-diketo-l-gulonic acid. Thus it was deduced that ascorbic acid is a reduced derivative of this lactone or 3-keto-l-gulonolactone. In order to determine whether the ring system, thus revealed, had a furanose or pyranose structure, methylated derivatives of ascorbic acid were studied. It was finally concluded that in solution the compound is the enolic form of 3-keto-l-lfurano-lactone.

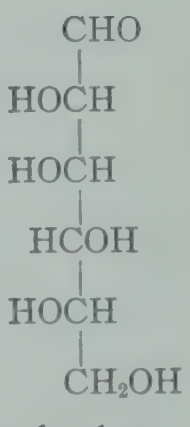
The sugars closely related to ascorbic acid, including l-glucose, are:



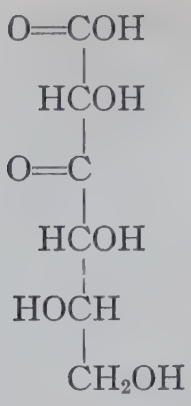
l-xylose



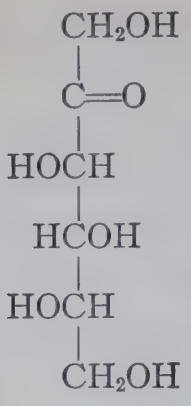
d-glucose



l-gulose

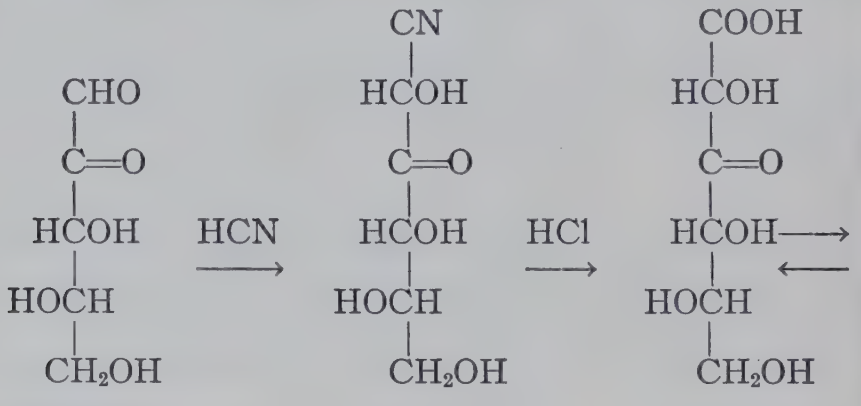


3-keto-l-gulonic acid

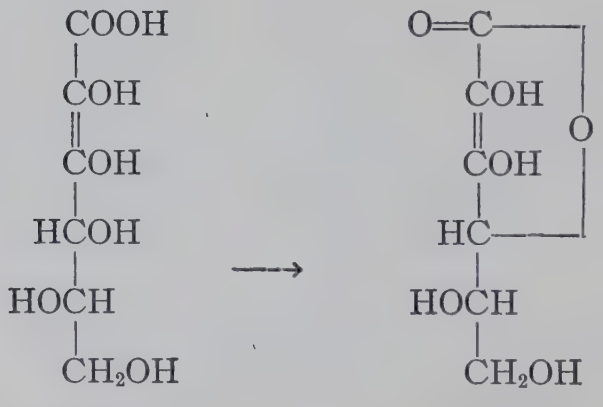


l-sorbose

Synthesis of Ascorbic Acid.—Before the structural formul represented above had been fully established, Reichstein and cowo ('33) announced the synthesis of ascorbic acid based on the follo series of reactions:



l-xylosone — β-keto-nitrile — keto-acid



keto-acid (enol form) — l-ascorbic acid

Both the d- and l- forms of ascorbic acid were synthesized, starting from d- and l-xylosone respectively. Almost simultaneously Haworth and Hirst and their collaborators (Ault et al., '33), by a procedure similar to that employed by Reichstein et al., also synthesized both the d- and l- forms of ascorbic acid.

The English workers reported that the reaction involving the addition of hydrogen cyanide goes to completion in the course of a few minutes and almost simultaneously the keto-acid is formed by hydrolysis in the acid solution. This intermediate product is readily changed into the vitamin in the presence of dilute HCl at 40 to 50° C. Micheel and Kraft ('34) described a third method for the synthesis. The principal reactions involved are:

$$\text{d-glucose} \xrightarrow{H_2} \text{sorbitol} \xrightarrow{\text{B. } xylinum} \text{l-sorbose} \xrightarrow{\text{phenylhydrazine}}$$

$$\text{l-sorbosone} \xrightarrow{Br_2} \text{2-keto-l-gulonic acid} \xrightarrow{HCl} \text{l-ascorbic acid.}$$

The 2-keto-l-gulonic acid is readily converted into l-ascorbic acid by formation of the ethyl ester which is then hydrolyzed into the vitamin through the action of weak alkali, or better, salts of weak acids or weak tertiary bases such as pyridine.

A synthesis by Reichstein and Grüssner ('35) which, according to King ('36, l.c.), has been used in the commercial synthesis of ascorbic acid, is:

$$\text{-glucose} \xrightarrow{H_2 \text{ and Pd}} \text{sorbitol} \xrightarrow{\text{B. } xylinum} \text{sorbose} \xrightarrow{\text{acetone}}$$

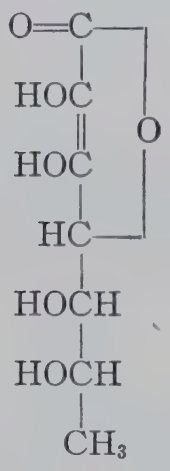
$$\text{iacetone sorbose} \xrightarrow{KMnO_4} \text{diacetone sorburonic acid} \xrightarrow{HCl}$$

$$\text{2-keto-l-gulonic acid} \xrightarrow{HCl} \text{l-ascorbic acid.}$$

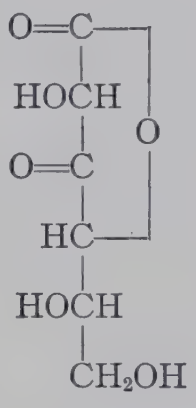
is stated that the yield is 20 to 30 per cent from sorbose. Thus in he space of two or three years brilliant chemical work reduced itamin C from the realm of recondite "little things" in nutrition to hat of a relatively cheap sugar of known chemical structure and eneral properties.

Compounds Physiologically Related to Ascorbic Acid.—According to Reichstein, Schwarz and Grüssner ('35), the essential condition or antiscorbutic activity is the d- configuration of the fourth carbon

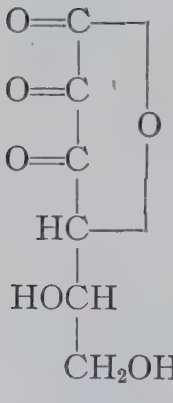
atom in ascorbic acid. Thus it was found that 6-methyl-l-arabo-3-k hexonic acid is active, having about one-fifth the activity of asco acid. d-erythro-3-keto-hexonic acid (isoascorbic acid), which di from ascorbic acid only in the steric arrangement of the groups the fifth carbon atom, has about one-twentieth to one-fiftieth activity of the natural vitamin (Dalmer and Moll, '33). Dehy ascorbic acid has approximately one-fourth the antiscorbutic pote of ascorbic acid in the reduced form. Structural relationships betw these compounds are as indicated:



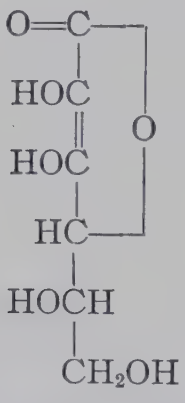
6-methyl-l-arabo-
3-keto-hexonic acid



d-erythro-
3-keto-hexonic acid



dehydro-ascorbic
acid



l-ascorbic acid
(vitamin C)

Some of the compounds which are inactive are: d-ascorbic aci d-gluco-ascorbic acid, and d-galacto-ascorbic acid.

The physiological significance of these relationships has not been determined, but in the case of dehydroascorbic acid it appears that the activity is due to the presence in blood of an enzyme capable of converting the oxidized vitamin into reduced ascorbic acid, which is the form in which the vitamin is believed to exist in the blood (Roe and Barnum, 36).

Chemical and Physical Properties of Ascorbic Acid.—In the relatively few years that ascorbic acid has been available in pure form, a considerable amount of information has accumulated concerning its chemical and physical properties. It is freely soluble in water and somewhat soluble in acetone and methyl, ethyl, and propyl alcohols. It is insoluble in ethyl ether and petroleum ether. Salts are formed with the various cations. For example, basic lead acetate was extensively used several years ago as a means of concentrating the vitamin. At present the sodium salt is frequently used for parenteral injections.

The compound forms colorless crystals whose melting point is 192° C. the rotation is +24° in water and +48° in alcohol. There is no mutarotation as is characteristic of somewhat related sugars. The absorption maximum is 260 mμ in water and 263-265 mμ in alcohol.

The first dissociation constant has been determined at various ionic strengths. According to Ball ('37) the true dissociation constant is pK 4.21. pK_2 is said to be 11.57.

Outstanding in properties is the oxidation-reduction of ascorbic acid. It is the reductant of a thermodynamically reversible system and sluggish in electro-motive activity. As stated by Ball, this sluggishness may account for the diversity of opinion with regard to its oxidation-reduction behavior. Borsook and associates ('37) claim that only the first oxidation, ascorbic acid ⇄ dehydroascorbic acid, is physiologically reversible and significant in its antiscorbutic action. It is interesting at least that ascorbic acid, in non-aqueous media, is almost completely without reducing action.

At pH 7.0 and below the vitamin is not autoxidizable, but this occurs in alkaline solutions and increasingly as the pH is raised (Barron, DeMeio and Klemperer, '36). This form of lability is of much physiological significance and it is plausible to expect that various factors, both *in vivo* and *in vitro*, greatly influence the oxidation-reduction of this substance. Among the metallic salts tested (Mn, Ni, Fe, Co, Ca, and Cu) copper is the only catalyst for the oxidation of ascorbic acid, its action being detectable in concentrations as small as 46 micrograms of copper per liter. The catalytic effect is completely inhibited by 0.001 M KCN, and to the extent of 80 per cent by mixtures of $CO:O_2$ of 95.5. This marked effect of copper

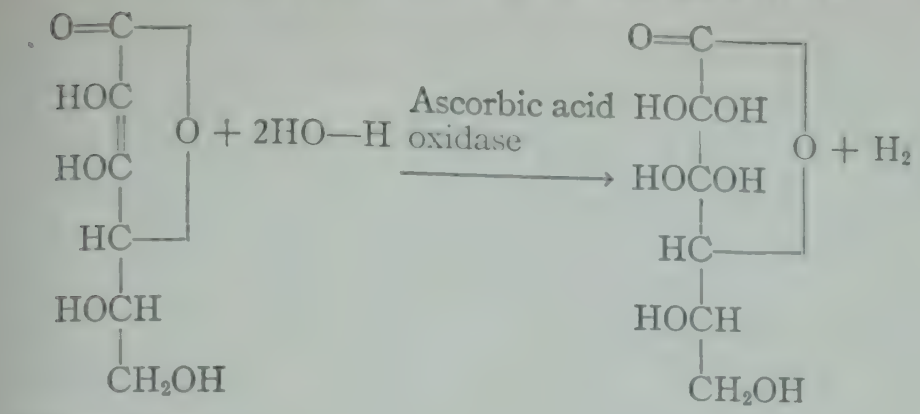
can be controlled by means of certain compounds such as m phosphoric acid, diethyldithiocarbamate, 8-hydroxyquinoline, g thione, and other substances which form copper complexes (Lyn Schultze and King, '37).

The oxidation product obtained with aqueous iodine under further oxidation with alkaline sodium hypoiodite to yield, quan tively, oxalic and trihydroxybutyric acids. Another name for the la substance is l-threonic acid. Ascorbic acid reacts with ozone, atoms of oxygen being added, with the formation of an alpha-ox derivative of 3,4-dimethyl l-threonate.

In both plant and animal tissues, enzymes play a prominent in the oxidation-reduction functions of ascorbic acid. The compou in its dehydrogenated state, is reduced in minced muscle, liver, kidney which has been properly buffered. Szent-Györgyi ('28, was the first to demonstrate the presence in plant tissue of an enzy which specifically catalyzes the oxidation of ascorbic acid. He poin out that this enzyme, in the absence of ascorbic acid, is with action on glutathione. However when the former is present latter is readily oxidized. Hopkins and Morgan ('36) amplified observation and found that when ascorbic acid and glutathione together in the presence of Szent-Györgyi's "hexoxidase," the la wholly protects the former from oxidation to dehydroascorbic ac In this system the glutathione is oxidized at a rate which, with same concentration of enzyme, is exactly similar to that when ascor acid is oxidized alone. Only when the reduced glutathione has pr tically disappeared from the system does the oxidation of ascor acid begin.

When ascorbic acid has been reversibly oxidized to dehydroascor acid, its reduction by pure glutathione is a very slow process; in the presence of the enzyme, under proper conditions, the reducti may be five times as fast as the oxidation induced by the same co centration of the enzyme without glutathione. Glutathione also co pletely protects ascorbic acid from oxidation by copper cataly From these data it appears that both ascorbic acid and glutathio the two most conspicuous and, so far as is known, the most act reducing substances in living tissues, play important roles in biologi oxidation-reductions.

Tauber, Kleiner and Mishkind ('35) have isolated an extrem powerful enzyme from the pericarp of the Hubbard squash, whi differs considerably from the one described by Szent-Györgyi. oxidizes ascorbic acid instantaneously and completely without t interaction of other catalytic agents. Molecular oxygen of the mosphere serves as hydrogen acceptor, and the oxidation process visualized as an introduction of two OH groups at the double bon



Reduced form of ascorbic acid

Oxidized form of ascorbic acid

It is of much interest that the oxidase appears to be absent from ammalian tissues (Tauber, '36-37), while at least the blood cells d plasma of humans and rats contain an enzyme that reduces hydroascorbic acid. The latter enzyme, it will be observed, has e opposite effect from the ascorbic acid oxidase of plant tissues. ucumbers contain the oxidase, but it is absent from the juice of ange, lemon, tangerine, and grapefruit. It is in part due to this sence of ascorbic acid oxidase that the antiscorbutic value is pre-rved on storage, but there is also a stabilizing effect of the relatively w pH of these fruits. Tauber takes issue with Sharp ('36) who ributes the rapid destruction of ascorbic acid in milk to the presence the oxidase. Tauber could not detect appreciable oxidase action milk. He attributes the ascorbic acid destruction to the presence traces of copper.

Visible light is said to have a markedly destructive action on the amin. Even light which passes through a milk bottle causes a id destruction. Whether this effect is related to the presence of pper or other catalysts is not clear.

In view of the above it is understandable that ascorbic acid is dily destroyed during the ordinary cooking of foods. However an inert atmosphere it is quite stable to moderate heat, although reaction might be alkaline. But in the presence of oxygen, truction is rapid and complete when the pH is above 7 and heat used. The vitamin is more stable to oxygen in acid media, hence undergoes less destruction in foods containing free acid such as us fruits, tomatoes, etc., than in less acid foods. Modern com-rcial processes of canning foods prevent marked ascorbic acid truction by removing much of the air from food before it is ilized in the cans by heat. The process of drying foods tends to roy much of the vitamin.

Chemical Methods of Estimating Ascorbic Acid.—Until biological assays were the only methods of estimating the vitam content of materials. In that year Tillmans and associates ('32, developed a chemical procedure based on the quantitative redu of an oxidation-reduction indicator, 2, 6-dichlorophenolindopheno

Dye Indicator Method: The essential features involve a pre nary extraction of the material to be assayed with trichloracetic and titration of the acid extract with the indicator. But as pointed by King ('36, l.c.) three hazards are particularly involved in titra of this type. These are: "(a) other substances may be present w reduce the titration reagent; (b) a portion of the vitamin ma present in the reversibly oxidized form, and (c) substances ma present which interfere with the reaction of either the oxidizin reducing agent."

Each year since the discovery of this method has seen the pub tion of numerous attempts to improve its usefulness, as well as to plant it by other procedures. Van Eckelen and Emmerie ('36) numerous papers, claim that preliminary precipitation with merc acetate is necessary for specificity in titration. They also insist preliminary treatment with hydrogen sulfide is necessary to re any oxidized vitamin present to the reduced form. Many work however, feel that these procedures are not advantageous. Rece metaphosphoric acid has been used successfully for the extrac of ascorbic acid. By this means ascorbic acid oxidase activity is hibited and the interference of copper is prevented. In brief, us information can be obtained by this method and in spite of var criticisms the procedure has not been superseded by anything m satisfactory for most purposes.

Furfural Colorimetric Method: This procedure, devised by ('36), is based on the determination of furfural formed by boiling acid extract of a tissue in which the ascorbic acid has been oxidi by passage through norite, with HCl alone and with HCl contain $SbCl_2$. It is claimed that the method is applicable to both plant animal tissues.

Microchemical Method: Glick ('35) has improved a microchem procedure described by Birch, Harris and Ray in 1933 which ma possible the extraction and titration of ascorbic acid with consider precision in amounts as small as 0.001 mg. of the substance. By method he and his associates have been able to determine quantitati the ascorbic acid content of histological sections of liver, adrenal, pophysis, and other tissues. Moreover they have been able to estim the concentration of ascorbic acid in various zones of the tissues, relative number of different cells in these zones, and the ascorbic content per cell.

Ascorbic Acid Estimation in Living Tissues: It has been claimed Rotter ('37) that the rate of decoloration of 2, 6-dichlorophenolin-phenol, injected into the sole of guinea pigs, is dependent upon e amount of ascorbic acid in the tissues. From his experiments on man subjects and guinea pigs Rotter concludes that a decoloration ne of about 5 minutes indicates saturation of the tissues with ascorbic id and that more than 10 minutes indicates deficiency.

Methylene Blue Method: Lund and Lieck ('36) found that if a all amount of ascorbic acid is added to a solution of methylene ue and the solution is exposed to strong light the color will com-etely disappear in 30 seconds. The decoloration is promoted by low H and the presence of sodium chloride. Estimation of serum ascorbic id is conducted with a stock solution of KH_2PO_4, 9 gm.; NaCl, 2 n.; and 0.004 per cent of methylene blue, in 100 cc. of water. It is ted that reaction to light is not brought about by glutathione, ergo-oneine, creatine, creatinine, urea, adenine, guanine, hypoxanthine, nthine, uric acid, cystine, phenol, and hemoglobin; nor do these in-bit the reaction.

Enzymatic Method of Estimating Ascorbic Acid.—It appears that e ascorbic acid oxidase method of Tauber, Kleiner and Mishkind 5, l.c.) is more specific than any of the chemical procedures. The ethod is based on the specific oxidizing property of their oxidase ascorbic acid. The technic consists in determining, by suitable pro-dure, the reducing power of a tissue extract before and after the ion of the enzyme. The difference is a measure of the amount of orbic acid present. The enzyme, obtained from the Hubbard squash, es not affect phenols, glutathione, cysteine, or adrenalin. This would m to be a promising method.

Biological Methods of Estimating Ascorbic Acid.—Owing to marked progress in chemical and enzymatic methods of estima-n, the biological procedures have passed their peak of usefulness h respect to ascorbic acid. There are needs, however, for the latter hnics, particularly as checks on the reliability of the former meth-. Coward ('38) has discussed in detail the various useful biological cedures.

In general, the biological method is based on a determination of minimum dose of a foodstuff which will just afford complete pro-ion of guinea pigs against symptoms of scurvy. Some of these s are tenderness and swelling of the joints, a tendency to sit ched up, and the assumption of a "face-ache" position of the head, ation of growth, and loss of weight. A special application of the hod is that devised by Höjer. It is based on the histological ex-nation of the teeth of guinea pigs restricted to appropriate test s. Since ascorbic acid deficiency causes marked and characteristic

changes in the teeth (see the subsequent chapter) the metho played a useful part in studies of ascorbic acid.

International Units.—Several years ago (1931) the Interna Vitamin Conference defined a unit of "the antiscorbutic Vitami as "the Vitamin C activity of 0.1 cc. of fresh juice of the le Harris and Ray ('33) found considerable variability in the po of lemon juice and stated that the average amount of ascorbic was such that 1 mg. of ascorbic acid contained 21 I. U. of vitam The mean concentration of ascorbic acid in lemon juice is about mg. per cc., or 0.064 mg. per 0.1 cc. At present one International is 0.05 mg. of l-ascorbic acid. Hence there is some discrepanc tween the present standard and the old.

State and Function of Ascorbic Acid in Plant Tissues.—Du the past few years considerable discussion has occurred over the of ascorbic acid in plant tissues. Several workers, including G and Pal ('37) and Levy ('36), hold to the view that some of vitamin is present in the combined state in certain plants. The cipal basis of this conclusion is the fact that an increase in the am of titratable ascorbic acid occurs on controlled heating of certain materials. However, Stone ('37), Mack ('36), and others have vincingly shown that the effect is entirely attributable to the ac of heat on ascorbic acid oxidase in such foods.

When plant tissues containing the oxidase are heated, under trolled conditions, the process inactivates the enzyme, thus preven a conversion of some of the vitamin to dehydroascorbic acid. S this latter product causes no reduction of 2,6-dichlorophenolindoph it is evident that food materials containing the enzyme would c less reduction of the dye if the oxidase were allowed to act on ascorbic acid, as happens in the processes of extraction which do employ heat or other procedures liable to inactivate the enzyme.

Considerable significance has been attached to the close correla between photosynthetic functions of plants and the content of asc acid in structures containing chlorophyll, on which photosynthes dependent. Randoin, Giroud and Leblond ('35) observed that gu pigs became scorbutic when their only possible source of ascorbic was the colorless portions of plants, but animals receiving the chl phyll-containing portions remained normal. Thus there seems to parallelism between the distribution of ascorbic acid and that chlorophyll. Giroud et al. have shown also that in the growth plants, ascorbic acid precedes the appearance of chlorophyll. H it would seem that synthesis of the vitamin is not dependent u the action of chlorophyll. It is possible, however, that ascorbic aci essential in photosynthesis.

It has been known for many years that sprouted grains are anti-scorbutic. The ascorbic acid is formed almost simultaneously with the initiation of sprouting and quickly reaches a high concentration in all rapidly growing parts of plants. It seems probable, therefore, that the vitamin is essential in the growth processes and before these can occur it may be necessary for ascorbic acid to be synthesized.

Practically all of the ascorbic acid in plants appears to be present in reduced form.

Species-Differences in the Ability to Synthesize Ascorbic Acid.—A source of confusion in the early experimental studies was the failure of rats to become scorbutic when restricted to a diet which caused death from scurvy in guinea pigs. Finally it became evident that a marked species-difference existed with respect to the need for this vitamin. It was shown that rats are able to synthesize the substance in considerable amounts, since scorbutic guinea pigs could be cured by feeding them small amounts of liver from rats restricted for many weeks to a diet free from the vitamin (Parsons, '20). Of the various vitamins known at present none are characterized by such large species-differences in requirements as in the case of ascorbic acid. In fact it appears that a majority of the animal kingdom is readily capable of synthesizing this factor. The only species known definitely to require a dietary source are man, guinea pigs, and monkeys. Rats, dogs, prairie dogs, pigeons, chickens, ducks, geese, turkeys, guinea fowl, and pheasants do not require the vitamin in their diet. The status of mice, rabbits, swine, and cattle in this regard is not clear. Kleiner and Tauber ('36) have observed that mice grow at a subnormal rate and show abnormal behavior when the diet is deficient in ascorbic acid. However, these latter symptoms eventually disappear. It appears that rabbits on an ascorbic acid-deficient diet fail to remain in good health but they do not develop typical scurvy (Findlay, '21). The data are confusing regarding swine. Hughes and associates ('28) claim that pigs thrive without any obvious source of the vitamin, but Zilva and coworkers ('24) state that they have produced scurvy in such animals. According to Thurston, Eccles and Palmer ('26), calves also apparently do not require ascorbic acid in the diet. It would be of interest to study the question of species-differences in other animals, particularly in the various sub-human primates. On the basis of present data it may be tentatively assumed that mice, rabbits, swine, and cattle need some ascorbic acid in the diet. It is possible that they are able to synthesize the vitamin to a limited extent.

Apparently there is no sharp distinction between different species as respects their needs for a dietary source of ascorbic acid. On the basis of body weight, guinea pigs seem to require proportionately

much more ascorbic acid than monkeys or children. It is evident the phylogenetic aspects of ascorbic acid synthesis and bodily quirement are in need of being further clarified.

Biosynthesis and Origin of Ascorbic Acid in Nature.—Var attempts to determine the mechanism of ascorbic acid formatio plants and certain animals have resulted in scarcely any clarifica of the question. Ray ('34) studied ascorbic acid formation at var stages in the germination of seeds. Various hexoses were used in culture medium and of these mannose seemed to serve as the m effective precursor, since the largest amount of ascorbic acid see to be formed when it was employed. The significance of mannose been further indicated by the various studies of Guha and Gh ('36) who have investigated ascorbic acid synthesis by *in vitro* *in vivo* experiments. They claim that ascorbic acid is formed w embryonic guinea pig tissue and rat liver, kidney, or spleen are in bated with mannose. Solutions of other hexoses were not effect Moreover they claim that parenteral injection of mannose causes increase of the ascorbic acid content of rats. But neither von Eu Gartz and Malmberg ('35) nor Kleiner and Tauber ('36, l.c.) w able to confirm these findings. Until the studies of Ray, and Guha a Ghosh are strengthened by more convincing evidence the conclus cannot be changed that neither the site of synthesis nor the precurs of ascorbic acid are known.

Studies have been made of the effect of various dietary imbalan and deficiencies on the concentration of ascorbic acid in tissues of t rat, it being assumed that such dietary manipulations might interf with the formation of this vitamin and thus suggest the nature of precursors and the site of its synthesis. Hopkins and Slater ('35) fou that during fasting, or on a diet very low in carbohydrate, the conte of ascorbic acid in the liver falls, while there is an increase in th of the intestines. They cautiously suggested that the vitamin is sy thesized in the intestinal epithelium and that in the absence of carb hydrate, protein or fat may serve as sources of the precursors. T hypothesis was flatly denied by Zilva ('36) on the grounds that was not supported by adequate evidence. Svirbely's ('36) conclusio tend to support those of Hopkins and Slater, but it would seem th Zilva's opinion also applied to his interpretations. Zilva reasons th the increased concentration of ascorbic acid in the intestinal w ". . . could just as well be assumed to be due to the transfer of t vitamin from the other parts of the body to the organ in questi . . ." On the basis of these studies, as well as those which cannot mentioned, owing to lack of space, it must be stated that scarcely an thing is known concerning the precursors of ascorbic acid and the s of its synthesis.

Distribution of Ascorbic Acid in Tissues.—Ascorbic acid appears o be present in all living tissues. Animals which do not require a ietary source of the vitamin synthesize it in relative abundance, thus ndicating that this substance is of fundamental significance in the netabolism of all living cells. The concentration tends to be highest n glandular tissues and lowest in muscular and fatty tissues. The rder of concentration is somewhat as follows: adrenals, pituitary, orpus luteum, thymus, pancreas, liver, spleen, testes, ovaries, brain, hyroid, submaxillaries, intestines, heart, kidneys, lungs, muscles, and dipose tissue.

The approximate ascorbic acid content of some human tissues, as etermined by Yavorsky, Almaden and King ('34), is given in the llowing tables:

ASCORBIC ACID CONTENT IN DIFFERENT AGE GROUPS
(Mg. per Gm.)

GE GROUP . . .	1–30 days	1–12 mos.	1–10 yrs.	11–45 yrs.	46–77 yrs.
O. OF CASES . .	11	9	11	17	19
drenal	0.581	0.525	0.550	0.393	0.230
rain	0.460*	0.189*	0.433*	...	0.110*
ancreas	0.365	0.304	0.225	0.152	0.095
ver	0.149	0.148	0.163	0.135	0.064
leen	0.153	0.112	0.157	0.127	0.081
idney	0.153	0.122	0.098	0.098	0.037
ng	0.126	0.057**	0.058	0.065**	0.045**
art	0.076	0.049	0.042	0.042	0.021
ymus	0.304	0.319	0.190	...	0.046*

* Average of two specimens only
**Average of six specimens only

PICAL INDIVIDUAL VARIATIONS IN ASCORBIC ACID CONTENT OF HUMAN TISSUES
(Mg. per Gm.)

AGE	ADRENAL	PANCREAS	LIVER	SPLEEN	KIDNEY	HEART
days . . .	0.913	0.237	0.244	0.126	0.200	0.076
ll-born . .	0.745	0.343	0.110	0.184	0.099	0.042
days . . .	0.059	0.056	0.078	0.018	0.047	0.020
mos. . . .	1.300	1.000	0.238	0.188	0.305	0.117
mos. . . .	0.084	0.080	0.068	0.048	0.034	0.017
yrs. . . .	1.030	0.274	0.234	0.161	0.145	0.078
yrs. . . .	0.191	0.087	0.114	0.124	0.067	0.049
yrs. . . .	0.076	0.177	0.276	0.272	0.040	0.053
yrs. . . .	0.033	0.022	0.029	0.023	0.022	0.027
yrs. . . .	0.027	0.022	0.018	0.012	0.011	0.017

DEPLETION OF ASCORBIC ACID FROM GUINEA PIG TISSUES

DIET	ADRENAL	LIVER	KIDNEY	N A
	mg. per gm.	*mg. per gm.*	*mg. per gm.*	
Basal diet + spinach	0.70	0.10	0.08	
Basal diet, ascorbic acid-free, 15 days (beginning to lose weight)	0.08	0.03	0.03	
Basal diet, ascorbic acid-free, 28 days (severe scurvy)	0.03	0.01	0.01	

It will be noted that marked age differences appear to Whether these are physiologically significant or only referab differences in nutritional status is not known. Yavorsky et al. that patients with a history of inadequate dietary ascorbic aci correspondingly low amounts of the substance in their tissues.

Biskind and Glick ('36), in an important series of histoche investigations, have estimated the distribution of ascorbic acid v certain organs, using a method sensitive to ± 0.001 mg. of the vit Their technic permits a study of various portions of an organ cording to their findings the pars intermedia of the pituitary co the highest concentration of any tissues on record, being abou times that of the adrenal cortex. Cow's corpus luteum contained 1.4 mg. of ascorbic acid per gm. of tissue when the organ was fully developed and the value fell to 0.3 mg. per gm. with regre Such studies are useful as means of indicating the functions of and their interrelationships. The general technic appears to be siderably more reliable than the silver nitrate stain for ascorbic in tissues.

Vitamin P.—The evidence concerning vitamin P does not wa much discussion. This principle was first described by Szent-Gy and associates in 1936 (Bentsáth, Rusznyák and Szent-Györgyi, According to these workers pure ascorbic acid is not effective i creasing the permeability of capillaries in scurvy. But, they extracts of Hungarian red pepper or lemon juice are beneficial fractionating lemon juice they were able to isolate a crystalline fla "citrin," which was responsible for the decrease in capillary per bility. Hence the name "vitamin P" was chosen for this factor. St of the substance in several different clinical conditions, as well scorbutic guinea pigs, led Szent-Györgyi and coworkers to con that experimental scurvy is due to a combined deficiency of asc acid and vitamin P.

However it is not generally conceded that the indispensability of the principle, vitamin P, is established. Zilva ('37) was unable to demonstrate any beneficial effect of this factor in delaying the onset of scurvy or the fatal termination of that disease in guinea pigs. Several observations in this country of the maintenance of apparently good health over long periods of time in guinea pigs receiving pure ascorbic acid and no source of vitamin P suggest further that the evidence is still in question regarding the necessity of a "vitamin P" for the maintenance of proper capillary permeability.

REFERENCES

King (1936). Physiol. Rev., *16*, 238.
Zilva (1928). Biochem. J., *23*, 1199.
Bezssonoff (1931). Bull. Soc. chim. biol., *13*, 950.
Smith and King (1931). J. Biol. Chem., *94*, 491.
Cox (1937). Science, *86*, 540.
Szent-Györgyi (1927). Biochem. Ztschr., *181*, 433.
Szent-Györgyi (1928). Biochem. J., *22*, 1387.
Zilva (1932). Biochem. J., *26*, 1624.
McKinnis and King (1930). J. Biol. Chem., *87*, 615.
King and Waugh (1932). Science, *75*, 357.
Svirbely and Szent-Györgyi (1932a). Nature, *120*, 576.
Svirbely and Szent-Györgyi (1932b). Nature, *129*, 690.
Tillmans, Hirsch and Hirsch (1932). Ztschr. Untersuch. Lebensm., *63*, 1.
Waugh, Bessey and King (1933). Proc. Soc. Exper. Biol. & Med., *30*, 1281.
Cox, Hirst and Reynolds (1932). Nature, *130*, 888.
Karrer, Salomon, Morf and Schöpp (1933). Biochem. Ztschr., *258*, 4.
Karrer, Schwarzenbach and Schöpp (1933). Helv. Chim. Acta, *16*, 302.
Karrer, Schöpp and Zehender (1933). Helv. Chim. Acta, *16*, 1161.
von Euler and Klussmann (1933). Ztschr. f. physiol. Chem., *219*, 215.
Micheel and Kraft (1933). Ztschr. f. physiol. Chem., *216*, 233.
Herbert, Hirst, Percival, Reynolds and Smith (1933). J. Chem. Soc., p. 1270.
Reichstein, Grüssner and Oppenauer (1933). Helv. Chim. Acta, *16*, 561, 1019; id., Nature, *132*, 280.
Ault, Baird, Carrington, Haworth, Herbert, Hirst, Percival, Smith and Stacey (1933). J. Chem. Soc., p. 1419.
Micheel and Kraft (1934). Naturwissenschaften, *22*, 205.
Reichstein and Grüssner (1935). Helv. Chim. Acta, *18* 608.
Reichstein, Schwarz and Grüssner (1935). Helv. Chim. Acta, *18*, 353.
Palmer and Moll (1933). Ztschr. f. physiol. Chem., *222*, 116.
Roe and Barnum (1936). J. Nutr., *11*, 359.
Hall (1937). J. Biol. Chem., *118*, 219.
Bullock, Davenport, Jeffreys and Warner (1937). J. Biol. Chem., *117*, 237.

Barron, DeMeio and Klemperer (1936). J. Biol. Chem., *112*, 625.
Lyman, Schultze and King (1937). J. Biol. Chem., *118*, 757.
Hopkins and Morgan (1936). Biochem. J., *30*, 1446.
Tauber, Kleiner and Mishkind (1935). J. Biol. Chem., *110*, 211.
Tauber (1936-37). Proc. Soc. Exper. Biol. & Med., *35*, 422.
Sharp (1936). Science, *84*, 461.
Van Eekelen and Emmerie (1936). Biochem. J., *30*, 25.
Roe (1936). J. Biol. Chem., *116*, 609.
Glick (1935). J. Biol. Chem., *109*, 433.
Rotter (1937). Nature, *139*, 717.
Lund and Lieck (1936). Nature, *137*, 784.
Coward (1938). "Biological Standardisation of the Vitamins." Baillie Tindall and Cox, London.
Harris and Ray (1933). Biochem. J., *27*, 580.
Guha and Pal (1937). Nature, *139*, 843.
Levy (1936). Nature), *138*, 933.
Stone (1937). Biochem. J., *31*, 508.
Mack (1936). Nature, *138*, 505.
Randoin, Giroud and Leblond (1935). Bull. Soc. Chim. biol., *17*, 1649.
Parsons (1920). J. Biol. Chem., *44*, 587.
Kleiner and Tauber (1936). Food Research, *1*, 399.
Findlay (1921). J. Path. & Bact., *24*, 454.
Hughes, Aubel and Lienhardt (1928). Bull. Kans. Agric. Exper. St *23*, 48.
Zilva, Golding and Drummond (1924). Biochem. J., *18*, 872.
Thurston, Eckles and Palmer (1926). J. Dairy Soc., *9*, 37.
Ray (1934). Biochem. J., *28*, 996.
Guha and Ghosh (1936). Nature, *138*, 844.
von Euler, Gartz and Malmberg (1935). Biochem. Ztschr., *282*, 39
Hopkins and Slater (1935). Biochem. J., *29*, 2803.
Zilva (1936). Biochem. J., *30*, 857.
Svirbely (1936). Am. J. Physiol., *116*, 446.
Yavorsky, Almaden and King (1934). J. Biol. Chem., *106*, 525.
Biskind and Glick (1936). J. Biol. Chem., *113*, 27.
Bentsáth, Rusznyák and Szent-Györgyi (1936). Nature, *138*, 798.
Zilva (1937). Biochem. J., *31*, 915.

CHAPTER

XVII

Nutritional Significance of Ascorbic Acid (Vitamin C)

N THIS DECADE of synthetic ascorbic acid and rapidly expanding nowledge of its physiological significance, it is somewhat difficult o understand how our ancestors were so slow in recognizing the reventives of scurvy—fresh fruits and vegetables. The fault was not tter lack of knowledge since Bachstrom in 1734, according to James ind (1753), positively asserted that fresh fruits and vegetables are alone effectual to preserve the body from this malady." But the rroneous theories of scurvy were intrenched and not easily changed, nce the concept of specific dietary deficiencies as causative factors n certain diseases did not exist except in a very vague fashion.

Perhaps the most readily available history of the disease is the dmirable book by Hess ('20). But the symptomatology of scurvy and ne great waste of life and health occasioned by that "calamity of ailors" were clearly set forth by Lind in 1753. Lind deserves the redit for having established, by many observations, that scurvy can e prevented or cured by the provision of fresh fruits or vegetables in ne diet. Although his dramatic experiments were made in 1747 it as not until 1795 that the British Navy began the regular adminis- ation of lime juice to the sailors and it was as late as 1865 that the ritish Board of Trade adopted this life-saving regulation.

Expeditions and armies, as well as sea-farers, have suffered great sses from scurvy. A notable instance was the voyage of Jacques artier in 1536 (Bigger, '24). While exploring the .St. Lawrence iver he lost 26 of his party from scurvy, but saved the remainder y means of a pine needle infusion recommended by the Indians of at region, as a specific remedy against scurvy.

But it was not until the present century that scurvy was studied animals. In 1907 Holst and Frölich found that guinea pigs become

scorbutic on a diet of oats and bran. The immediate advances m by this important discovery will not be recounted here since they accessible in various reviews (Sherman and Smith, '31; Waugh, ' The synthesis of ascorbic acid, which has made it available in p form and at low cost, and the development of chemical methods estimation, have contributed to the accumulation of many studies the physiological significance of this nutrient. A discussion of vari aspects of this subject will be the purpose of this chapter.

Effects of Ascorbic Acid Deficiency.—In humans a deficiency ascorbic acid scarcely ever occurs unaccompanied by other ina quacies or dietary imbalance. These tend to produce variability in symptoms referable to scurvy but in general it may be stated t scurvy has the following characteristics: The adult loses weight, anemic, weak, and short of breath. The gums become swollen, ble easily, and frequently ulcerate. The teeth loosen and may drop o Necrotic areas in the jaw bones may occur. Hemorrhages into t mucous membrane and skin are characteristic. Blue-black spots velop in the skin after trivial injury, or they may occur spontaneous The ankles become edematous, and in severe cases there develops hard boardlike condition of the skin and subcutaneous tissues. Ne ous symptoms of various types may appear. Children and infants a fretful, anemic, and without appetite. They fail to grow satisfa torily and exhibit vague evidences of illness.

These general symptoms, alone, are only vaguely suggestive the role of ascorbic acid in bodily processes. Since the histopatholog effects of the deficiency are more important for this purpose, great attention will be given to them here. The present information h been critically discussed by Wolbach ('37) who has contributed mu of the data on this subject. In brief, ascorbic acid functioning is pa ticularly concerned with cells of mesenchymal origin. This nutrie is necessary for the formation of all intercellular substances havi collagen, or collagen-like substance, as their basis. Thus the chara teristic hemorrhages of scurvy are attributable to the inability of t body to produce intercellular material. The latter is necessary prevent separation of the single layer of endothelial cells which for the walls of blood capillaries. It is the cementing substance whi holds these cells together. This intercellular material constitutes t foundation of all fibrous structures, the matrices of bone, denti cartilage, and all non-epithelial cement substance, including that the vascular endothelium.

This general concept of ascorbic acid in relation to specific typ of cells was reached through studies of human scurvy, which ma have been accompanied by other dietary defects, and experiment scurvy in guinea pigs. The latter probably was unassociated with a

ther dietary defects. It will be profitable, therefore, to describe the rincipal pathologic findings in such structures as are notably affected y ascorbic acid deficiency. These are teeth, bones, and blood vessels.

Teeth: As early as 1919 it was shown experimentally by Zilva and Vells that radical changes in tooth structure are associated with deciency of the antiscorbutic nutrient. Howe (1919) also investigated ental changes in this deficiency, being primarily concerned with udies of the cause of dental caries. However, it was the extensive nd classic work of Höjer ('24) which revealed in detail the pathologic ffects of ascorbic acid deficiency on the teeth. A summary of the anges he observed, stated in his own words, is as follows:

1. The gradual change of the odontoblast layer. This seems to be a re sign of scurvy and one of the earliest changes to set in. The odontoasts assume another shape, grow shorter, more rounded, and show nother arrangement, the normal regular, creeper-like formation makes ay to a layer, which in some places curves in towards the center of the ulp and is soon split through the cells secreting a hard tissue between emselves, so that the syncytial character of the cell layer does not plainly ppear. The odontoblasts have been transformed into osteoblasts placed bone canals. . . .

2. The amorphous calcification of the predentine.

3. Widening of Tomes' canals in the dentine before the onset of urvy.

4. New formation of bone instead of dentine. This bone is first lying a thin layer inside the calcified predentine, but soon extends reticularly wards the center of the pulp. This bone has a spongy, porous character.

5. Dilation of vessels and in early stages hyperemia; sometimes hemorages in the pulp.

6. Atrophy and resorption of pulpa tissue, pulpa cells, the newly rmed bone and the old dentine. This resorption which, as everywhere bone formation, will surely be found also with scurvy in all stages, pears more distinctly after all the new bone formation in the final stage s stopped, and may proceed so far, that in place of pulpa tissue there seen nothing but some large hollows filled with fluid.

7. In the healing of the scurvy—at an earlier stage—reorganization the pulpa bone into irregular dentine, osteodentine, with bone canals d dentinal canals.

8. In a scurvy which is latent all through or very much mitigated, nen at least half the amount of antiscorbutic needful to an individual is ovided—forms which are the most common in man—the progress is nilar, though not so pronounced and presents pictures that differ conerably less than the normal. If the antiscorbutic dose provided is -0.7 of the minimum protective dose, an irregular dentine is formed th Tomes' canals in most places, but in the lingual part of the pulp re are found in some symmetric places ridges of a hard tissue, with character of bone and lacking dentinal canals. In this pulpa bone as

well as in the newly formed irregular dentine, there are canals of Have type and isolated bone corpuscles which may be considered to cons of transformed odontoblasts.

With an antiscorbutic dose of 0.8 or more of the minimum protecti dose, the picture here described is changed so far that there is no pul bone, but all the newly formed hard tissue in the pulpa consists of oste dentine, or even dentine (the first formed layer).

Thus Höjer believed that the characteristic and basic effects o the deficiency was a development of active osteoblasts from odonto blasts, resulting therefore in the production of bone instead of denti This view was not questioned by Wolbach and Howe ('26) but the were unable to confirm the dental changes except by alternate feedin of deficiency and complete diets. When their diets were very deficien formation of "osteodentin" or pulp bone, as reported by Höjer, di not occur.

Wolbach and Howe's diet consisted of autoclaved soy beans 50 rolled oats 29, dried milk powder (Klim) 10, brewer's yeast 4, butte fat 5, calcium carbonate 1, and sodium chloride 1, parts respectively Filter paper was included as roughage. Guinea pigs were reared from weaning to 3 or 4 years of age when they were given this diet supplemented with 8 cc. daily of orange juice. Teeth of guinea pigs restricted to the deficient ration underwent dramatic changes. It was noted that after 7 days the odontoblasts had begun to separate from the dentin; also, their form had begun to change. After 12 days extensive separation had occurred and vacuoles were to be seen between the dentin and odontoblastic layer. This separation was practically complete at the end of 14 days. In addition, the Tomes fibrils, processes which extend from the odontoblasts and permeate the Tomes canals of the dentin, had been ruptured. At this stage, if the animal is given orange juice (ascorbic acid), and is killed after 72 hours, newly formed dentin is present. After 7 days of recovery the newly formed dentin is more extensive, and is irregularly traversed by Tomes fibrils, which arise through regeneration and are not continued into the old dentin. As stated by Wolbach ('37, l.c.), "In guinea pigs, dentin formation is resumed in volume and rapidity suggestive of the jelling of a liquid material between pulp and old dentin."

A theory to account for the action of ascorbic acid on dental structures has been advanced by Fish and Harris ('34). It considers that the nutrient is primarily needed to promote the functional activity of the formative cells, i. e., odontoblasts, ameloblasts, osteoblasts, etc. The concept of Höjer is rejected that odontoblasts change their function and revert to osteoblasts. They emphasize that in severe degrees of ascorbic acid deficiency during tooth development the

ameloblasts (enamel formers) may actually disappear or become badly impaired. This causes serious defects in the enamel formed at the time of the deficiency. Since the defects are irreparable the tooth remains in constant danger of decay at the site of these weak areas.

Bones: Gross and histological effects of deficiency observed in bones are basically similar to those in other structures and tissues, namely, a failure in the proper functioning of intercellular material. Rarification of the long bones occurs and this, naturally, is frequently accompanied by fractures, especially at the epidiaphyseal junctions in growing bones. In young guinea pigs deprived of ascorbic acid the first demonstrable bone change is seen at the epidiaphyseal junctions and it is characterized by an increase in the number of osteoblasts applied to the cartilage columns. As Wolbach ('37, l.c.) has written, "Formation of cartilage and bone matrices ceases, and the osteoblasts become elongated, assume the shapes of fibroblasts and migrate toward the diaphysis. Here these cells become surrounded by liquid, presumably a deficient product of continued activity toward matrix formation, and give rise to an apparent region of edematous connective tissue at the ends of the diaphysis, the Gerüst Mark (framework narrow) of German authors."

As the deficiency progresses resorption of bone matrix occurs. This causes profound changes in the epiphyseal cartilage. Since periosteal bone formation also ceases, trabeculae of the spongy bone fail to maintain a sufficient degree of attachment with the epiphyseal cartilage. Consequently fractures occur between the epiphysis and diaphysis. Movement occurs between epiphysis and diaphysis, resulting in a "Trümmerfeldzone." The periosteum becomes separated from bone cortex owing to the continued proliferation of osteoblasts of the periosteum. Hemorrhages occur in these tissues, forming, in addition to the cellular changes, a characteristic pattern. These changes are most striking at the costochondral junctions. Here, as in other bony structures, the osteoblasts increase in number but no intercellular substance is formed. During this period there is resorption of bone salts, thus accounting for the rarification.

Dramatic reparative processes quickly take place when ascorbic acid is restored to the diet. Newly formed intercellular material appears within 24 hours as the osteoblasts resume their role in the formation of this material. Capillary formation, which is essential to the deposition of bone salts, is resumed and other restorative processes continue.

These histopathologic evidences of bone changes are substantiated by X-ray examination and gross inspection. Park and associates ('35) have made important contributions to our information on the clinical aspects of ascorbic acid deficiency, particularly with reference to its

recognition by means of X-ray changes. These studies indicate t insufficient attention has been given to the effects of scurvy on bo In view of the apparently widespread lack of adequate ascorbic a in the dietaries of both children and adults it seems probable t careful study of the bones of such persons would reveal defi abnormalities attributable to a deficiency of this vitamin.

Blood vessels: It has been emphasized that the characteristic hem rhages in various parts of the body are referable to mechanical we ness of the blood vessels and their supporting structures. This, turn, is related to the reduced cohesion of the vascular endotheliu In ascorbic acid deficiency the "cement substance" is less effective producing cohesion between the endothelial cells. Hence bleedi from the gums, and extravasations of blood into the skin and oth tissues are attributable to the loss of this intercellular material nec sary for the maintenance of tissue integrity. Since changes in t fragility of capillaries are somewhat amenable to objective measu ment, various tests of "capillary fragility" have been employed detect subacute degrees of ascorbic acid deficiency. These will briefly discussed subsequently.

Other Tissues and Structures: Bessey, Menten and King ('3 have extended our knowledge of the pathologic changes in the orga of scorbutic guinea pigs. In the adrenals there occurs, in severe scurv a translocation of fat which, in the normal gland, is present on in the cytoplasm and is distributed as a film on the periphery granules. This fatty material is unsaturated. It is transferred in drople to the vessels which transport it to the medulla. The capillaries ma be loaded with freed fat and if the animal survives, the fat ma disappear from the cortical area. The cholesterol likewise disappea from this area. Coincident with the extreme lipid alteration in th adrenals, extensive fatty degeneration occurs in foci irregularly di tributed in the myocardium. This begins by a shifting of the fat fro the dark or anisotrophic band into the light band where it increas until droplets are formed. These become fused with loss of morpho ogy of the myocardial cell. This, they infer, constitutes an irreversib change and forms the beginning of the typical patches of fatty d generation like those seen in diphtheria. Similar fatty changes a observed in the skeletal muscles. There is also fatty infiltration the liver in varying degrees of scurvy, and it is so marked that i the terminal stages the cells of an entire lobule are almost entire composed of fat. The infiltration is associated with fatty degener tion which they regard as probably the primary lesion.

In severe scurvy they observed extreme degenerative changes i the testes, with death of the germinal epithelium and spermatozo They also described an edematous condition of the periportal conne

ive tissues of the splenic septa and the media of the small arterioles f the lungs. In the lung, such vessels occasionally presented the ppearance of obliterative endarteritis. They interpret these changes s primarily alterations in the colloidal state of the collagen, as postu- ated by Wolbach.

Effects of Ascorbic Acid in Massive Doses.—Although pure scorbic acid has been available for about 4 years at reasonably low rices, the effects of this substance in massive doses have not been dequately studied. The present evidence indicates, however, that no arm is done although the quantities ingested may be many times ne approximate daily requirement. Demole ('34) found that up to gm. of the pure vitamin per kilogram in one dose were tolerated ithout symptoms by animals. Also, no histological changes were bserved in the kidneys, liver, heart, and lungs. Likewise no changes r symptoms were noted in animals given 12 gm. per kilogram over period of several days.

After the tissues become saturated with the substance excesses ppear to be readily and quickly excreted unchanged in the urine. As nown by Everson and Daniels ('36) working with children of pre- chool age, retentions of ascorbic acid parallel the ingestion level only p to approximately 7.5 mg. per kilogram of body weight. Ingestions f 10 to 12 mg. per kilogram did not appear to augment the level f retention.

It is probably true that the body can excrete excesses of ascorbic id without difficulty over long periods of time. However, informa- on is needed regarding the effects of continuous high dosage of this bstance. Is there any similarity between renal clearance of ascorbic id and that of creatinine, xylose, urea, and other substances which ave been investigated? Are there any species-differences in ascorbic id tolerance, especially as related to animals which can synthesize e substance as compared with those which are unable to synthesize ? Does the continuous ingestion of ascorbic acid in large amounts use any degree of structural injury in the kidneys, liver, or other gans and tissues of the body? These are some of the questions which ture investigations should attempt to answer.

Factors Affecting the Retention and Excretion of Ascorbic cid.—Various data indicate that retention of ascorbic acid will not cur when the body is "saturated" with this substance. "Unsaturated" sues, however, have remarkable capacities for retention of the vita- n. Schultzer ('36) reports the interesting cases of three scorbutic tients who required 7.0, 9.5, and 14.4 gm. respectively of ascorbic d to "fill" the tissues before additional ingestions of the substance ulted in significant renal excretion. By comparison, the daily adult quirement appears to be about 40 mg. All the evidence suggests that

the kidneys have a rather definite ascorbic acid threshold and th levels of ingestion beyond a certain limit, determined by various fa tors, result in rapid urinary excretion of the excess. Probably the blo level of ascorbic acid is the determining factor in its excretion sin the value is usually below 0.4 mg. per cent in cases of ascorbic ac deficiency (Taylor, Chase and Faulkner, '36) and when liber amounts of the vitamin are being ingested the level is 2 to 3 tim that amount. According to van Eekelen ('36) saturation of the o ganism coincides with a certain level in the blood which is about 1 mg. per 100 ml. When this level is exceeded excretion in the urin results. Thus the retention seems to be somewhat similar to that certain common freely diffusible blood constituents.

The ability of guinea pig and human tissues to retain ascorbic aci depends in some measure on factors other than the content of th substance already in the body. For example, Hawley and associate ('36) found that normal persons saturated with ascorbic acid excrete almost 100 per cent of daily test doses of the pure vitamin when am monium chloride was administered with it. However the same dosag of vitamin, accompanied by sodium bicarbonate, was followed by a excretion of only approximately 50 per cent of ascorbic acid in th urine. When guinea pigs were given sufficient amounts of sodiu bicarbonate to produce a highly alkaline urine, the ascorbic acid, fe in doses of 10 mg. per animal per day, was retained in significantl greater amount in the adrenals and liver than in the case of guine pigs given the vitamin alone or with ammonium chloride. It ma be significant also that Jacobsen ('35) noted a better retention o ascorbic acid in guinea pig tissues when cabbage was used instead o the pure vitamin. For example, the adrenals contained 18 mg. per cen of ascorbic acid when each animal was given 20 mg. of ascorbic aci per day for 3 months. When cabbage was fed, the value was 78 mg per cent. We have no explanation for this apparent superiority o natural foods over synthetic material.

Acetylsalicylic acid (aspirin) (Daniels and Everson, '36) and ethe (Bowman and Muntwyler, '37) also cause increased excretion o ascorbic acid. Although the effect appears to be of short duration there is justification for assuming that regular use of aspirin migh necessitate an increased intake of ascorbic acid to balance the height ened urinary excretions.

Finally, the body's ability to subsist without injury during periods of deficient ascorbic acid intake is greatly limited. As shown by O'Hara and Hauck ('36), normal adult women restricted to an experimental diet furnishing approximately 5 mg. of the vitamin per subject per day soon showed evidence of ascorbic acid depletion. After only one or two days on such a diet the daily urinary output of the

vitamin fell to about 15 mg. After 4 weeks on the diet one subject developed swollen gums and other evidences of scurvy. These disappeared promptly when ascorbic acid, furnished by orange juice, was given. Approximately 2 to 3 gm. of the vitamin were required to resaturate the subjects. Thus the evidence accumulates that even temporary dietary restrictions of ascorbic acid, such as may occur in the treatment of certain diseases, *viz.*, gastric ulcers, may lead to dangerous consequences.

Relationships between Ascorbic Acid Intake and its Content in Milk.—Selleg and King ('36) supplemented the diets of lactating women with ascorbic acid and determined the effect on the ascorbic acid content of their milk, using the dye indicator titration method. The following table shows typical results:

Average Daily Ascorbic Acid Supplement	Number of Cases	Ascorbic Acid (mg. per cc. of milk)						
		Initial Values	Values Following Supplementation					
			Days Post Partum					
		3 to 6	5	6	7	8	9	10
one . . .	17	0.053	0.056	0.058	0.060	0.066	0.064	0.064
0 mg. . .	19	0.056	0.067	0.070	0.068	0.071	0.072	0.073
2 mg. . .	17	0.055	0.065	0.070	0.076	0.077	0.078	0.081

It was found that supplementing the diet with ascorbic acid causes slow and limited rise in the antiscorbutic value of the milk. This ached a maximum value which is not increased by excessive dietary take of the vitamin. The excess is rapidly excreted in the urine ithout disturbing the lactation level.

Selleg and King, reviewing the data of other investigators, conude that the normal level of ascorbic acid in human milk is much gher than in cow's milk, the latter having a content of approxiately 0.02 to 0.025 mg. per cc. Milk rapidly loses its antiscorbutic lue. The average value for raw (unpasteurized) market milk is proximately 0.01 mg. per cc.

Riddell and coworkers ('35) have studied the effect of ascorbic id supplements on the ascorbic acid content of cow's milk and have viewed the literature on the subject. By means of both chemical d biological methods the milk of 74 cows fed a dry ration plus

silage contained 25.8 mg. of ascorbic acid per liter. The value f milk from cows on green pasture was 26.5 mg. One would infer fro these data that the cow is able to synthesize ascorbic acid, since t dry ration would have provided very little of this substance. It al appears that the content found represents the maximum level mammary secretion and that an excess of ascorbic acid in the ratio is destroyed or excreted in the urine. It is generally accepted th pasteurization destroys practically all of the ascorbic acid in milk.

If the normal ascorbic acid requirement of a human infant may estimated from the concentration in human milk, this may be co sidered, on the basis of an intake of 21 ounces daily, to be about mg. per day. But, as shown by Selleg and King, the milk of certa women may contain as little as 0.019 mg. of ascorbic acid per Infants dependent upon such milk for all their ascorbic acid probab would not receive enough. The significance of optimal ascorbic ac intake in lactating women is indicated by the findings of Yavorsk and coworkers, already cited, that the tissues of certain infants a very deficient in ascorbic acid. There is no evidence that human ti sues, in the embryonic or infantile state, can synthesize ascorbic aci

Functions of Ascorbic Acid in the Body.—In the previous cha ter it was shown that ascorbic acid may be essential in photosynthes although it is formed in sprouting seeds before chlorophyll can b detected. The present opinion is that the substance is an essenti participant in cellular respiration and that a deficiency of this nu trient may disturb various enzyme systems. The fact that protozo bacteria, moulds, lichens, and algae give a positive ascorbic acid r action, when tested with acetic acid-silver nitrate, suggests that vit min C is distributed throughout all forms of life (Bourne and Alle '35). If this is true, the very existence of living protoplasm may b dependent upon the presence of ascorbic acid.

The studies of Barron and associates (Barron and Barron, '36 are suggestive of an important role of ascorbic acid in blood formatio and the maintenance of a physiologic level of erythrocytes in th circulating blood. When ascorbic acid was injected intravenously int rabbits simultaneously with cobalt sulfate, polycythemia failed t appear. But when the ascorbic acid was withheld it appeared in or 7 days. Moreover, ascorbic acid tended to reduce polycythemi Cobalt seems to produce polycythemia through the inhibition of th respiratory function of immature cells, thus resulting in their extru sion into the general circulation as mature non-respiring cells. Henc a normal function of ascorbic acid may be the regulation of the leve of red cells in the circulating blood even though cobalt is not preser in abnormal amounts.

On the basis of evidence cited thus far ascorbic acid appears t

have essential roles in (a) cellular respiration and in (b) the formation and regulation of intercellular matrix. Whether these functions are mutually exclusive is not known but it seems plausible to assume that they are not. It may be that intercellular matrix formation and regulation is dependent upon the oxidation-reduction role of ascorbic acid. As summarized by Stotz and associates ('37) ascorbic acid can function as a hydrogen transfer agent in oxidation-reduction reactions because of: "(a) its great sensitivity to reversible oxidation, (b) its occurrence in practically all actively respiring tissues of plants and animals . . . , and (c) a certain amount of evidence that the vitamin can act directly upon recognized metabolites and enzymes *in vitro*."

As pointed out elsewhere (Chapter XVI), glutathione protects ascorbic acid against oxidation by oxygen and ascorbic acid oxidase. The mechanism of this reaction is that the oxidized ascorbic acid reacts with glutathione, thus causing the reformation of ascorbic acid (reduced) and the production of oxidized glutathione, this reaction being much faster than the oxidation of ascorbic acid. Ascorbic acid is regarded, therefore, as a coenzyme for the glutathione oxidation.

The available *in vitro* and *in vivo* studies do not clarify the role of ascorbic acid in oxidation-reduction much further than is stated here. Phillips, Stare and Elvehjem ('34) found that ascorbic acid deficiency in guinea pigs produced relatively little effect on the rate of oxygen uptake of liver. Stolz and associates reported the contrary, stating that the oxygen uptake of liver was very noticeably augmented with the onset of scurvy. The problem is one for further study.

Requirements for Ascorbic Acid.—Adequate evaluations of ascorbic acid requirements are dependent upon objective methods of determining the health status in relation to ascorbic acid ingestion. Defects in the determination of either the health status or the amount of ascorbic acid ingested make it impossible to evaluate the requirement. Although this simple truth is obvious it will bear emphasis.

Chemical methods of assaying foods for ascorbic acid have been made fairly reliable in recent years, and it appears that some of the tests of subacute ascorbic acid deficiency may be used to give indications of ascorbic acid requirements, such as capillary resistance, simple blood analysis, and "saturation" tests, the latter of which involve titration of urinary ascorbic acid before and after the ingestion of a standard quantity of ascorbic acid. But these are based on plausible assumptions. Since the amount of this vitamin in the blood of non-scorbutic persons is higher than in those with scurvy, it has been suggested that the content of ascorbic acid in the blood may be used as a measure of the health status of a person with respect to

this nutrient (Farmer and Abt, '35; and others). The saturation te originated by Harris and Ray ('35), serve a useful purpose in the di nosis of subacute ascorbic acid deficiency, but their use in estimat requirements for the vitamin, other than for the prevention of scur are not sufficiently refined for unequivocal acceptance. It is theref impossible to discuss ascorbic acid requirements except in terms broad generalities.

On the basis of capillary resistance methods, Göthlin ('34) a others have concluded that the average adult needs about 25 mg. ascorbic acid daily to prevent the slightest objectively determined p scorbutic ill effects. On the basis of this figure Harris and associa ('35) have presumed that adults are not receiving even the minin requirements if the urinary excretion of ascorbic acid, by titrati falls below 10 to 15 mg. per day. Van Eekelen ('36, l.c.), by mea of a saturation test procedure which may not be very reliable, h concluded that an adult weighing approximately 70 kg. requi about 60 mg. of ascorbic acid per day in order to remain in go health. This value has been confirmed by Heinemann ('36) by mea of a similar procedure. Since it appears that the dietaries of ma adults who appear to be in good health furnish not more than mg. of ascorbic acid per person per day it seems probable that mg. is an amply safe figure for the adult requirement, since it twice the approximately minimum requirement.

Harris and associates ('35, l.c.) tentatively concluded that t requirements of infants and children, calculated per unit of bo weight, are greater than those of an adult. This conclusion is su ported by Everson and Daniels ('36, l.c.) but there are not yet a data which unmistakably indicate the requirements for this age grou Everson and Daniels found that ascorbic acid retentions in childr 3 to 5 years of age paralleled the ingestions only up to 7.5 mg. thereabout per kilogram of body weight, but higher levels of ingesti caused no increase in the retention. Certainly it would seem th infants should receive at least one-third as much ascorbic acid adults. Thus the minimum level for infants should not be less tha 10 mg. Children over 9 years of age probably should receive as mu ascorbic acid as adults.

Without doubt pregnant and lactating women need considerab more ascorbic acid than normal adults. Neuweiler ('35) has describ ascorbic acid excretion studies on non-pregnant, pregnant, and lact ing women which he conducted in order to estimate the requiremen during pregnancy and lactation. As would be expected, it was nec sary for the pregnant and lactating women to ingest considerab larger amounts of the vitamin in order to maintain an excretion lev regarded as indicative of adequate vitamin intake. The data do n

offer sufficient evidence to warrant definite commitments regarding the requirements in absolute terms of ascorbic acid. However it was fully demonstrated that the needs during lactation are even greater than in pregnancy. On the basis of these data and others in the literature, it is probably safe to assume that pregnant women should ingest 75 to 125 mg. of ascorbic acid per day and that lactating women should take 100 to 150 mg. per day.

Data on the ascorbic acid requirements of guinea pigs may be of some value in considerations of human needs. According to Dann and Cowgill ('35), ". . . the vitamin C requirement of the guinea pig is directly proportional to the body weight, and is almost exactly 1 cc. of lemon juice per 100 gm." They conclude from their data that young, rapidly growing guinea pigs do not require a proportionately greater amount of ascorbic acid than the adult. This is at variance with the opinions cited above concerning human needs. Without doubt the usual conclusion applies here that further investigation is needed.

Estimation of Sub-acute Degrees of Ascorbic Acid Deficiency. —The discussion of dietary requirements has emphasized the need for reliable and useful means of estimating mild degrees of ascorbic acid deficiency. Various procedures have been proposed but none are fully satisfactory. The methods include measurement of capillary fragility, X-ray examination of bones, concentration of ascorbic acid in blood, urinary excretion of ascorbic acid, and "tissue saturation" or ascorbic acid "tolerance." The latter method is considerably more promising than the others although the blood level of this substance is undoubtedly a useful criterion of mild deficiency.

The simplest tests are those involving capillary fragility. They are based on the well-known fact that blood vessels are weakened by the lack of ascorbic acid and the degree of fragility seems to correspond somewhat to the degree of deficiency. Among the more recent improvements of the test is that described by Wright and Lilienfeld ('36). Göthlin ('33) and Dalldorf ('33) have contributed valuable information on capillary fragility. The general procedure used by Wright and Lilienfeld, and Göthlin, is the compression of arm veins by means of a rubber cuff around the arm in order to constrict the capillaries. The number of petechiae formed with a given pressure is regarded as a measure of the degree of ascorbic acid deficiency. Dalldorf's method is based on the production of negative pressure outside the capillaries by means of a suction cup pressed against the skin. Weld ('36) and others have criticized capillary resistance tests stating that they obtain great variations in response which in many cases certainly are not related to ascorbic acid deficiency.

X-ray examination of bones is not promising, although the bone

changes in scurvy are characteristic. The method is more expens and probably less sensitive to slight degrees of deficiency.

Abt, Farmer and Epstein ('36) believe the concentration of ascor acid in blood appears to be closely related to the state of nutrition respects ascorbic acid.

The urinary excretion of ascorbic acid alone is regarded by Abba Harris, Ray and Marrack ('35, l.c.), and others, as a good indicat of the ascorbic acid nutritional status, but they believe the use of t doses of ascorbic acid gives a much more objective and reliable in cation of the nutritional status as respects this vitamin. It is genera agreed that the basis of this test is sound and that the results do ag with such other criteria as can be used. In the method of Harris a associates the ascorbic acid content of 24-hour samples is determin by titration. The subject is restricted to his habitual diet and given test dose orally of pure ascorbic acid. For adults of average size 6 mg. are recommended. The amount for children is less, dependi on their approximate body weight. Following this the urinary exc tion is again determined and from the results a curve is construct which indicates the rate and amount of ascorbic acid excretion. subjects well nourished with respect to ascorbic acid the curve ris rapidly to a high level and quickly decreases to the basal level. persons whose bodies are deficient in the substance the curve ris slowly to a low level and gradually recedes to a level which tends be a little higher than the basal, depending upon the degree of ascorb acid deficiency.

The data of Ralli and associates ('37) are instructive in this r gard. Normal adult subjects given 100 mg. of pure ascorbic acid b intravenous injection excreted more than 40 per cent within 3 hou Persons restricted to ascorbic acid-low diets excreted 11 per cent und similar conditions. Clinical subjects with scurvy excreted less than per cent of the test dose.

Ascorbic Acid in Immunological Reactions.—Numerous invest gations of the role of different dietary essentials in bodily resistan to disease have shown that various types of nutritional deficiencies a accompanied by a lowering of the immunological defenses. Howeve there has been scarcely any agreement on the question of direct rel tionships between dietary essentials and immunological processes. Ce tainly in the case of vitamin A there is no convincing evidence th the formation of antigens or antibodies, for example, is directly a fected by a deficiency of this nutrient. The role of this vitamin resistance to infection is related to the maintenance of healthy muco surfaces, as pointed out elsewhere (Chapter XIII). Hence its functio is indirect.

But in recent years it has been indicated that ascorbic acid ma

tually play a direct role in bodily resistance to disease. For instance, larde and Thompson ('35) suggest that complement may contain corbic acid as a component since the thermolability of both substances is similar. After histamine shock the content of complement nd ascorbic acid in blood was reduced. Injection of ascorbic acid led a significant rise in complement. Marsh ('36) has reported that the omplement" complex in the blood of the guinea pig disappears or ffers reduction in titer when ascorbic acid is withdrawn, completely partially, from the diet. Moreover, he states that the complement er can be restored to normal by means of a diet rich in ascorbic id. Horgan's ('36) observations are in complete agreement with those Marsh. In addition, Horgan noted that guinea pigs rarely died om the effects of intra-cardiac puncture when the diet contained enerous amounts of ascorbic acid.

These claims are at variance with some of the older data. Zilva 19) did not detect any difference in the content of natural antiodies in normal guinea pigs and those fed a scorbutic diet. Hamurger and Goldschmidt ('22) observed no appreciable difference complement titer between normal and scorbutic guinea pigs and ildren. But the studies of Koch and Smith ('24) suggested that ere is a connection, although seemingly obscure, between ascorbic id and complement.

Recently the role of ascorbic acid in the function of complement s been greatly clarified by the studies of Ecker and associates ('38) ho, using improved methods of titrating complement, have demonated a marked decrease in complement parallel with the reduction ascorbic acid of the serum. Administration of ascorbic acid to rbutic guinea pigs increased the complement titer. Moreover, the dition of ascorbic acid to scorbutic serum increased the titer to rmal. An increase of the ascorbic acid content of the serum beyond mg. per 100 cc. did not increase the titer. Apparently the vitamin nctions, in relation to the complement titer, through its reducing operties. Presumably information will be available soon on the relan of ascorbic acid to complement in humans.

As pointed out by Perla and Marmorston ('37), in a review on role of ascorbic acid in resistance, skin reactivity to drugs, such arsphenamine, is greatly diminished in guinea pigs depleted of orbic acid. This is true also in the case of skin hypersensitiveness poison ivy. Sulzberger and Oser ('34) found that large doses of orbic acid reduced and inhibited the susceptibility of the guinea 's skin to experimental sensitization with neoarsphenamine. The ount required was larger than that necessary to protect against rvy.

These evidences of a direct role of ascorbic acid in the immunologic

defenses of the body were supported by the studies of Jusatz ('3 whose work is discussed in a full-page editorial by the *Journal the American Medical Association* (Editorial, '37). Jusatz claims t large doses of ascorbic acid injected intravenously into rabbits ha a stimulating effect on specific antibody production. It is stated th 100 mg. of ascorbic acid added to each immunizing dose of horse p tein caused an average 5 to 7 fold increase in specific precipitin p duction in rabbits whose power to form specific antibodies had be greatly reduced by restriction to routine laboratory diets subjected prolonged pressure cooking at 120° C. Unfortunately this mode preparing experimental rations makes it impossible to learn the natu of the specific dietary factors involved in the reduction of speci antibody formation. It is perhaps significant that such marked sponses to ascorbic acid were noted in rabbits since the animals a normally capable of synthesizing the vitamin. The work of Jusatz given credence by the studies of Hochwald ('35), who has report that ascorbic acid appears to play a role in allergic and anaphylact phenomena.

Ascorbic Acid in Relation to Infectious Diseases.—The prese information, although meager, forms a significant background f the discussion of ascorbic acid in relation to infectious diseases, esp cially in relation to rheumatic fever, pulmonary tuberculosis, dip theria, and pneumococcus sepsis. Ascorbic acid undoubtedly plays significant part in the prevention and cure of all of these condition but there is no unequivocal evidence that this nutrient has a specif role in the prevention or cure of any of them. Some of the mo significant observations as respects each of these infectious processe as well as some other diseases, will be considered forthwith.

Rheumatic Fever: Various nutritional factors have been propose as etiological agents of rheumatic fever. For example, it has been su gested that oxalic acid plays a role (Loeper, '32) since in certain rheu matic states it appears that there is an excess of oxalates in the bloo There is scant basis for the theory of oxalate excess as an etiologic factor in this disease.

Rinehart, Conner and Mettier ('34) found that chronicall scorbutic guinea pigs infected with beta streptococci develope arthropathy with pathological changes which they regarded as simil to rheumatic fever and rheumatoid arthritis. Also chronic scurvy alo appeared to produce this syndrome. In view of these findings an the fact that rheumatic fever tends to be a disease of malnourished an poorly housed children of northern climates (Swift, '31; Paul, et a '34), Rinehart ('35) suggested that rheumatic fever may be the resu of the combined action of ascorbic acid deficiency and infection. I acute rheumatic fever the ascorbic acid level of the blood plasma wa

ound to be almost uniformly lowered (Rinehart, et al., '36). This one is not convincing evidence that ascorbic acid plays a unique le in rheumatic fever, since infectious diseases in general seem to crease the requirement for this nutrient, thus lowering the blood vel when the intake is not adequate. Sendroy and Schultz ('36), well as others, have criticized the views of Rinehart and associates. hey have pointed out that a significant percentage of rheumatic ver cases appear to be unaccompanied by ascorbic acid deficiency. oreover, administration of this vitamin in doses approximately twice e probable normal requirement has not resulted in cure of the dis-se. But, as Rinehart has pointed out, an adequate study of the fect of high intake of ascorbic acid in rheumatic fever has not been ade. We are without sufficient information concerning variability ability to utilize ascorbic acid to conclude that present evidence oves the absence of a causal relation between systemic ascorbic acid sufficiency and rheumatic fever.

As recently shown by Taylor ('37), scurvy in guinea pigs leads valvulitis and myocarditis with acute and subacute inflammatory ci. No organisms were found in many of the severest lesions. How-er, gram-positive cocci or bacilli were present in about one-half the diseased hearts. This study certainly suggests that opponents of nehart's views have not established their claims that disturbances rheumatic fever, as respects ascorbic acid, are of no greater signifi-nce than those of similar character observed in other infections. rther investigation may show that ascorbic acid insufficiency is not specific factor, but it seems very improbable that this nutrient is thout some effect in the prevention of rheumatic fever.

Pulmonary Tuberculosis: In recent years it has been indicated that berculous persons require a larger intake of ascorbic acid in order to aintain a normal ascorbic acid balance. The studies of Heise and artin ('36) are important in this respect. They estimated the urinary orbic acid excretion of 54 patients with mild, moderately advanced, d far advanced tuberculosis, using the Tillmans titration method. e diets of all were presumably adequate. The data were classified 4 arbitrary groups in the order of their magnitude. The number of ients with active tuberculosis was then determined for each group, d with striking results. Six patients excreted only 0 to 5 mg. daily. e of these had active tuberculosis and it was believed that the sixth d some degree of activity. Fourteen excreted 5 to 8 mg: daily. Active ection was present in 9 of the 14. Twenty-one excreted 8 to 14 mg. ly and only 7 of the 21 showed activity. Finally, in the group ex-ing over 14 mg. daily only 1 of the 13 showed activity.

The significance of these data was amplified by estimations of rbic acid "tolerance." Heise and Martin administered 4 ounces

daily of orange juice, containing about 55 mg. of ascorbic acid, the same patients. This amount was sufficient to cause a significa increase in the amount excreted by normal persons ingesting an dinary diet presumably containing an adequate amount of ascor acid. Eight of 10 cases showing active tuberculosis did not respond w increased excretion of the vitamin, but 14 inactive cases showed significant rise in the excretion level. The data show that between and 140 mg. of ascorbic acid were required daily by patients w active tuberculosis for the maintenance of a normal urinary level the vitamin. In one patient 200 mg. daily were not sufficient to cau a normal excretion level. The contrast of these figures with the 20 40 mg. daily of ascorbic acid required by a normal adult is strikin It is not yet clear whether the high rate of ascorbic acid destruction tuberculosis represents a specific requirement, or one generally ass ciated with chronic infectious diseases.

The data of other workers indirectly support these findings. F instance, Steinbach and Klein ('36) claim that daily injections ascorbic acid increased the tolerance of tuberculous guinea pigs repeated large doses of tuberculin. Also, scorbutic guinea pigs fe tuberculous sputum are more susceptible to ulcerative intestinal lesion of tuberculosis than in normal animals (McConkey and Smith, '3 Greene, Steiner and Kramer, '36).

However, there appears to be no experimental basis for the assump tion that large quantities of ascorbic acid will increase the resistance o normal persons or animals to tuberculous infection. In support of th Heise and Martin ('36) found that large daily parenteral injections o ascorbic acid failed to protect guinea pigs injected with virulen tubercle bacilli. This plan of study should be extended, since it i possible that the infecting dose of organisms was too high. Also th use of large doses of ascorbic acid should be investigated with refer ence to survival of animals subjected to various degrees of infection.

Diphtheria: As early as 1920 it was observed that latent scurvy i infants is associated with a relatively high incidence of nasal diphtheria Hess ('20, l.c.) attributed it to a local immunizing effect of the anti scorbutic vitamin on mucous membranes. At present there is incon trovertible evidence that ascorbic acid plays a significant role in pro tection against diphtheria toxin. Indirect evidence of this was afforde by Bessey, Menten and King ('33, l.c.) who found that in sever scurvy, heart muscle develops patches of fatty degeneration similar t those seen in diphtheria.

It is neither possible nor necessary to review all the work on thi subject. It suffices to mention only a few of the many investigations Prominent among these are the contributions of King and associates For instance, King and Menten ('35) fed guinea pigs abundant, pro

ective, and sub-protective amounts of ascorbic acid and gave them ubcutaneous injections of diphtheria toxin in various doses below the minimum lethal amount. Animals partially depleted of their ascorbic cid reserves, but showing no signs of scurvy, survived about half as ong as animals with normal reserves receiving the same dosage of liphtheria toxin. Menten and King ('35) noted that the ascorbic acid-epleted animals, injected with the toxin, developed diffuse hyper-lastic arteriosclerosis in lungs, liver, spleen, and kidneys. Such animals lso showed degeneration of the islets of Langerhans, which was asso-iated with high blood sugar and low glucose tolerance. The findings f Pfleger and Scholl ('37) give added significance to this report. In iabetic patients who were not given insulin, the administration of scorbic acid had no effect on the sugar content of blood or urine, but he combustion of acetone bodies was favorably influenced. When the issues were saturated with ascorbic acid the action of insulin was oticeably intensified, so that the carbohydrate metabolism could be egulated with smaller amounts of insulin. They state that all diabetic atients, irrespective of the modification of the sugar metabolism, were mproved as respects general condition, disappearance of fatigue, and ncreased vitality, by the administration of ascorbic acid.

When diphtheria toxin and ascorbic acid are mixed *in vitro* there a loss of reducing capacity proportional to the time of contact, and ne toxin loses its lethal effect after a certain interval (Jungeblut and wemer, '35; Polónyi, '35). Greenwald and Harde ('35) confirmed nis observation and stated that contact of ascorbic acid with antitoxin r toxin-antitoxin mixtures causes no loss of antitoxic potency.

Otto ('36) and other workers have investigated the uses of ascorbic cid in the treatment of diphtheria, as well as its metabolism in this nfectious disease. Otto found that the retention was especially great hen in cases of toxic diphtheria from 500 to 700 mg. of ascorbic acid as administered daily by intravenous injection. Patients to whom rge doses of the vitamin were not given excreted very small amounts the urine, indicating that the requirements in this disease are much igher than normal. But the administration of ascorbic acid, as a nerapeutic measure, does not appear to affect the time of disappear-nce of the diphtheric membrane and of the elevated temperature, nor oes it influence the number of fatalities. It was noted, however, that favorable influence was exerted on hemorrhages, particularly nose-eeds. According to Otto, a definite therapeutic value can be ascribed it only in cases of diphtheric hemorrhages. But, as King and asso-ates have emphasized, there is a wide zone of ascorbic acid deficiency, ithout the appearance of scurvy, where physiological processes are terfered with and the body is more sensitive to injury from toxins. he evidence suggests unequivocally that ascorbic acid should be fed

in generous amounts in diphtheria, as a means of preventing a de ciency of this nutrient in the tissues, if not as a definite therapeu agent.

Pneumococcus Infection: As in the case of rheumatic fever, p monary tuberculosis, and diphtheria, pneumonia also appears to cau a large increase in the ascorbic acid requirement. Bullowa and ass ciates ('36) found that in the high-fever stages of pneumonia, tiss depletion of ascorbic acid occurs, as estimated by the "saturatio test.

In summary, the evidence suggests that the ascorbic acid requir ments are augmented by all diseases in which an elevated temperatu occurs.

Ascorbic Acid in Relation to Cataract.—Although numero papers have been published on the relation of ascorbic acid to catara formation and in particular the metabolism of lens, it is impossib to draw final conclusions on the subject. Dietary factors other tha ascorbic acid certainly affect the lens. Lactose excess and riboflavi deficiency, in the rat, as discussed elsewhere, are reported to cau cataracts.

Josephson ('35) states that the administration of 15 to 300 mg. c ascorbic acid to patients with cataract caused marked improvemen and within a week of treatment mature cataracts became sufficientl transparent to permit examination of the eye grounds and even perm some vision. Cataracts caused by poisoning with dinitrophenol wer said to respond very rapidly to treatment with ascorbic acid, and othe toxic effects of the drug were also ameliorated. Bellows ('36) repor confirmation of the observations of others in respect to cataract forma tion in rats fed large amounts of galactose. He states that ascorbic aci given with the galactose delays the onset of changes in the lens. Len of galactose-fed animals contained less ascorbic acid than norma Also, yeast, which is rich in glutathione but devoid of ascorbic aci delayed the appearance of cataracts. The latter observation is signif cant since Bakker ('36) states that the transparency of the lens is no dependent upon its content of ascorbic acid and that a normal rate o respiration is possible when the amount of this vitamin is low. John son ('37) also has published evidence which indicates that ascorbi acid has no direct bearing on the etiology of cataract in guinea pigs.

It may be concluded that there is not sufficient evidence to warran generalizations regarding the role of ascorbic acid in lens metabolism particularly as it concerns cataract formation. The foregoing data ar suggestive of the need for further investigation of this subject.

Ascorbic Acid in Relation to Gastrointestinal Ulcers.—For sev eral years it has been suspected by a significant number of worker that gastrointestinal ulcers are in some instances caused by chroni

scorbic acid insufficiency. There is scarcely any reason to believe, owever, that ascorbic acid deficiency is generally related to such dis- rbances. Smith and McConkey ('33) noted that guinea pigs suffering om prolonged partial deficiency of ascorbic acid tended to develop eptic ulcers which were believed to be similar to those seen in human bjects. Hirsch ('35) reports the occurrence of peptic ulcers, previously iagnosed as neuroses, in two adolescent boys. He believes that ascorbic id is essential in the dietetic management of such cases. According Stepp ('36), bacteria of the coli and paratyphoid types destroy scorbic acid so that depletion from this cause might be a contribu- ry cause of peptic ulcers.

Although there is not adequate basis for any suggestions of a sig- ficant causal relationship between ascorbic acid insufficiency and strointestinal ulcers, it should be emphasized that the type of diets hich have been used in treating such conditions generally contain ry little ascorbic acid. This most certainly would be expected to re- rd the healing of lesions and interfere with the general physiological nctions. As shown by Archer and Graham ('36), 6 out of 9 patients ith gastric and duodenal ulcers were in the sub-scurvy state, owing parently to the use of customary bland diets which did not supply is nutrient. Lazarus ('37) reports a high incidence of ascorbic acid ficiency in patients with hematemesis and melena who had been ven typical dietary treatment. Gratifying remission of bleeding was tained in several cases that were treated with pure ascorbic acid.

Ascorbic Acid in Relation to Anemia.—There is evidence that n utilization is defective when the body's reserves of ascorbic acid e depleted. That anemia occurs in scurvy is well known, but it has en attributed by some to general undernutrition and hemorrhage, ough increased blood destruction has been suggested as an additional ctor. Mettier, Minot and Townsend ('30) state that in adults with urvy the pronounced anemia often encountered can be promptly ieved by providing foods rich in ascorbic acid. There is marked iculocytosis and rapid regeneration of blood. Neither large doses of n nor the substance potent in pernicious anemia appear to accom- sh these effects in the scorbutic state. The efficacy of ascorbic acid promoting normal hematopoiesis is further indicated by the studies Dunlop and Scarborough ('35), who reported marked blood re- neration after administration of ascorbic acid to a scorbutic subject ose diet contained only 10 mg. of iron per day. The nature of the ation to anemia is not known.

Ascorbic Acid in Relation to Addison's Disease.—The normally gh content of ascorbic acid in the adrenal glands, and the conclusive dence that its presence in any tissue is in some manner associated th an oxidation-reduction system, suggest that depletion of this

substance in the tissues may contribute to the occurrence of metabol disturbances of a specific character. The prominence of the adren glands as sources of epinephrine and cortin (perhaps several differe sterols or steroids of much physiological importance), as well as a ascorbic acid-rich tissue, in species which require a dietary source c ascorbic acid and those which do not, naturally has led to reflection o the significance of ascorbic acid in these glands.

By means of the urine titration method, Wilkinson and Ashfor ('36) found ascorbic acid deficiency in three cases of Addison's disease There was some increase in urinary excretion of the vitamin followin reported oral dosage in large amounts. When therapy was discontinue the increased excretion fell rapidly to values characteristic of the con trol period. In these few cases it was claimed that the degree of asco bic acid depletion paralleled the severity of the disease. They expresse the view that depletion of ascorbic acid may be a feature of the disease or it may be an entirely non-specific index to the extremely low state o health of the patient. Whether there is an increased need for ascorbi acid or an increased destruction of it in Addison's disease cannot b stated on the basis of present evidence. As has been noted above, ther is increased destruction of ascorbic acid in febrile conditions, but i Addison's disease fever is not a prominent feature.

Siwe ('35) found a high tolerance of ascorbic acid in patients with Addison's disease. The oral administration of 600 mg. daily did no increase the urinary excretion. He states that ascorbic acid therapy de creases pigmentation but without relieving the symptoms of the disease It has been reported that ascorbic acid reduced pigmentation in th case of Addison's disease in a 12-year-old boy (Harnapp, '36). Wilkin son and Ashford noted a decrease in the pigmentation of two patient who had been given liberal doses of ascorbic acid intramuscularly However, they hesitated to ascribe the effect to ascorbic acid since th patients had been given adrenal cortex extract. The present meage evidence merely suggests that pigmentation, of the kind associate with Addison's disease, and perhaps certain physiological conditions are related to a deficiency of ascorbic acid. Further studies of this sub ject should be made.

REFERENCES

Lind (1753). "A Treatise on Scurvy." Sands, Murray & Cochrane, London
Hess (1920). "Scurvy Past and Present." J. B. Lippincott & Co., Philadelphia.
Biggar (1924). "The Voyages of Jacques Cartier." Publications of the Public Archives of Canada, No. 11, Ottawa.
Sherman and Smith (1931). "The Vitamins." Am. Chem. Soc. Monograph, 2nd ed., New York.

UGH (1934). J. Chem. Ed., *11*, 69.
OLBACH (1937). J. Am. Med. Assoc., *108*, 7.
JER (1924). Acta Paediat., Suppl., *3*, 8.
OLBACH and HOWE (1926). Arch. Path. & Lab. Med., *1*, 1.
H and HARRIS (1934). Phil. Trans. Roy. Soc. London, *223B*, 489.
RK, GUILD, JACKSON and BOND (1935). Arch. Dis. Childhood, *10*, 265.
SSEY, MENTEN and KING (1933). Proc. Soc. Exper. Biol. & Med., *31*, 455.
MOLE (1934). Biochem. J., *28*, 770.
ERSON and DANIELS (1936). J. Nutr., *12*, 15.
HULTZER (1936). Acta med. Scandinav., *88*, 317.
YLOR, CHASE and FAULKNER (1936). Biochem. J., *30*, 1119.
T EEKELEN (1936). Biochem. J., *30*, 2291.
WLEY, FRAZER, BUTTON and STEPHENS (1936). J. Nutr., *12*, 215.
OBSEN (1935). Skand. Arch. Physiol., *72*, 259.
NIELS and EVERSON (1936). Proc. Soc. Exper. Biol. & Med., *35*, 20.
WMAN and MUNTWYLER (1937). Proc. Soc. Exper., Biol. & Med., *35*, 57.
HARA and HAUCK (1936). J. Nutr., *12*, 413.
LLEG and KING (1936). J. Nutr., *11*, 599.
ODELL, WHITNAH, HUGHES and LIENHARDT (1935). J. Nutr., *11*, 47.
URNE and ALLEN (1935). Nature, *136*, 185.
RRON and BARRON (1936). Proc. Soc. Exper. Biol. & Med., *35*, 407.
OTZ, HARRAR, SCHULTZE and KING (1937). J. Biol. Chem., *120*, 129.
ILLIPS, STARE and ELVEHJEM (1934). J. Biol. Chem., *106*, 41.
RMER and ABT (1935). Proc. Soc. Exper. Biol. & Med., *32*, 1625.
RRIS and RAY (1935). Lancet, *i*, 71.
THLIN (1934). Nature, *134*, 569.
BASY, HARRIS, RAY and MARRACK (1935). Lancet, *ii*, 1399.
INEMANN (1936). Biochem. J., *30*, 2299.
CWEILER (1935). Klin. Wchnschr., *14*, 1793.
SN and COWGILL (1935). J. Nutr., *9*, 507.
IGHT and LILIENFELD (1936). Arch. Internal Med., *57*, 241.
THLIN (1933). J. Lab. & Clin. Med., *18*, 484.
LDORF (1933). Am. J. Dis. Child., *46*, 794.
LD (1936). J. Pediat., *9*, 226.
, FARMER and EPSTEIN (1936). J. Pediat., *8*, 1.
LI, FRIEDMAN and KASLOW (1937). Proc. Soc. Exper. Biol. & Med., *36*, 2.
DE and THOMPSON (1935). Compt. rend. Acad. d. sci., *200*, 1425.
SH (1936). Nature, *137*, 618.
GAN (1936). Nature, *137*, 872.
A (1919). Biochem. J., *13*, 172.
IBURGER and GOLDSCHMIDT (1922). Jahrb. f. Kinderh., *100*, 210.
H and SMITH (1924). Proc. Soc. Exper. Biol. & Med., *21*, 366.
ER, PILLEMER, WERTHEIMER and GRADIS (1938). J. Immunology, *34*, , 39, 45; ib., J. Biol. Chem., *123*, 351, 359.
A and MARMORSTON (1937). Arch. Path., *23*, 543.
YBERGER and OSER (1934). Proc. Soc. Exper. Biol. & Med., *32*, 716.

Jusatz (1936). Ztschr. f. Immunitätsforsch. u. exper. Therap., *88*, 4
483.
American Medical Association, Editorial (1937). J. Am. Med. Assoc., *1*
713.
Hochwald (1935). Zentralbl. f. inn. Med., *56*, 769.
Loeper (1932). Nutrition, *2*, 151.
Rinehart, Conner and Mettier (1934). J. Exper. Med., *59*, 97.
Swift (1931). Am. Heart J., *6*, 625.
Paul, Harrison, Salinger and De Forest (1934). Am. J. Med. Sci., *1*
300.
Rinehart (1935). Ann. Internal Med., *9*, 586.
Rinehart, Greenberg and Christie (1936). Proc. Soc. Exper. Biol. & Me
35, 350.
Sendroy and Schultz (1936). J. Clin. Investig., *15*, 369.
Taylor (1937). Lancet, *i*, 973.
Heise and Martin (1936). Proc. Soc. Exper. Biol. & Med., *34*, 642.
Steinbach and Klein (1936). Proc. Soc. Exper. Biol. & Med., *35*, 151.
McConkey and Smith (1933). J. Exper. Med., *58*, 503.
Greene, Steiner and Kramer (1936). Am. Rev. Tuberculosis, *33*, 585.
Heise and Martin (1936). Proc. Soc. Exper. Biol. & Med., *35*, 337.
King and Menten (1935). J. Nutr., *10*, 129.
Menten and King (1935). J. Nutr., *10*, 141.
Pfleger and Scholl (1937). Wien. Arch. f. inn. Med., *31*, 219.
Jungeblut and Zwemer (1935). Proc. Soc. Exper. Biol. & Med., *32*, 12
Polónyi (1935). Wien. med. Wchnschr., *85*, 685.
Greenwald and Harde (1935). Proc. Soc. Exper. Biol. & Med., *32*, 11
Otto (1936). Klin. Wchnschr., *15*, 1510.
Bullowa, Rothstein, Ratish and Harde (1936). Proc. Soc. Exper. Bi
& Med., *34*, 1.
Josephson (1935). Science, *82*, 222.
Bellows (1936). Arch. Ophthalmol., *16*, 762.
Bakker (1936). Graefe's Arch. Ophthalmol., *136*, 166.
Johnson (1937). Biochem. J., *30*, 1430.
Smith and McConkey (1933). Arch. Internal Med., *51*, 413.
Hirsch (1935). Monatschr. f. Kinderh., *63*, 429.
Stepp (1936). Munchen. med. Wchnschr., *83*, 1119.
Archer and Graham (1936). Lancet, *ii*, 364.
Lazarus (1937). Brit. Med. J., *ii*, 1011.
Mettier, Minot and Townsend (1930). J. Am. Med. Assoc., *95*, 1089.
Dunlop and Scarborough (1935). Edinburgh Med. J., *42*, 476.
Wilkinson and Ashford (1936). Lancet, *ii*, 967.
Siwe (1935). Klin. Wchnschr., *14*, 1311.
Harnapp (1936). Monatschr. f. Kinderh.. *66*, 213.

CHAPTER

XVIII

Chemical Nature of Thiamin (Vitamin B_1)

E STUDIES of Lunin (1881), referred to in an earlier chapter apter II), brought clearly to the attention of physiologists the ence of a specific problem concerning the nature of the substances ch were lacking in a diet composed of purified proteins, carbohy-es, fats, and inorganic salts, without which normal nutrition could be attained. The essential repetition of his experiments by Pekel-ng (1905) and by Hopkins (1906) has already been discussed. ewise, the studies of Takaki (1887), which resulted in the demon-ion that beriberi could be eradicated from the Japanese Navy by a er dietary reform, and the epoch-making discovery of Eijkman 7) that the cardinal symptoms of beriberi could be induced in s by restricting them to polished rice, his postulation that this se was caused by a toxin which could be neutralized by a sub-ce present in rice polishings, and the correction of this error by man's successor, Grijins (1901), have been previously discussed. een the years 1913 and 1916 McCollum and his coworkers onstrated that, in addition to their "fat-soluble A," young rats ired another factor which they called "water-soluble B." They ested that the new factor was identical with the antiberiberi sub-e.

1926 Smith and Hendrick showed that the water-soluble factor of dual nature. This important discovery was utilized immediately oldberger (1926) in the extension of his researches on pellagra, ypothesis being that the heat-stable factor, demonstrated by Smith Hendrick, was the antipellagra dietary factor. Thenceforth the table component of the "B-complex" became known in England tamin B_2, and in America, as the P-P factor (Goldberger) and as vitamin G. Likewise the English workers adopted the term

vitamin B_1 for the more heat-labile, antiberiberi factor, but wor
in America tended to designate it as vitamin B, or the antineu
vitamin.

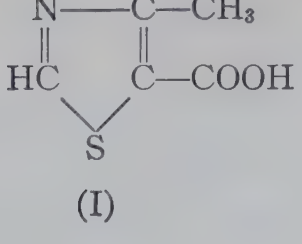
Nomenclature of Vitamin B.—In the older literature the foll
ing names have been employed to designate the substance which
vented or cured polyneuritis, the analog of human beriberi, in b
or mammals: Activator (Schaumann), vitamine (Funk), oryza
(Suzuki), antiberiberin (Tsuzuki), torulin (Edie), eutonine (Ab
halden), antineuritin (Hofmeister), water-soluble B (McCollum
Kennedy), vitamin B (Drummond), antiberiberi vitamin (Fu
Jansen), antineuritic vitamin (Mitchell), vitamin B-P (Salmon),
vitamin F (Sherman and Axtmayer). In the more recent literat
vitamin B_1 (Vitamin Committee of Medical Research Counc
aneurin (Jansen), and thiamin (Williams) are used. Aneurin see
to be the preferred name in Europe, whereas in America, thiamin
thiamin chloride (bromide, sulfate, etc., depending upon the a
radical combined with the base), is preferred. Commercial prepa
tions of the crystalline vitamin have been introduced under such nam
as Betabion, Betaxin, Betanerva, etc. The multiplicity of names cau
confusion and is deplorable. Thenceforth, in this book the na
thiamin will be employed, it being understood that the hydrochlor
is the substance referred to. There is adequate precedent for this usa
since the Council on Pharmacy and Chemistry of the American Me
cal Association is in favor of the name thiamin as a common desig
tion for vitamin B_1 (American Medical Association, '37), and it h
been adopted by the American Association of Biological Chemists a
the American Institute of Nutrition.

Isolation and Identification of Thiamin.—The early chemical
search on the isolation and identification of thiamin was marred
several instances by over-enthusiasm and lack of discretion in the p
mulgation of premature and uncritical work. Advancement was sl
owing in part to the lack of dependability in much of the chemi
information published from 1911 to about 1920, and the inherent di
culty of the problem. By 1911 Funk ('12) had shown that the fac
is soluble in water, alcohol, and acidified alcohol, is dialysable, re
tively stable in the presence of strong acids, and precipitable by ph
photungstic acid. Funk's insight placed the substance in the class
pyrimidine bases which has been substantiated, as further discussi
will show. Vedder and Williams' ('13) studies suggested further
pyrimidine-like nature.

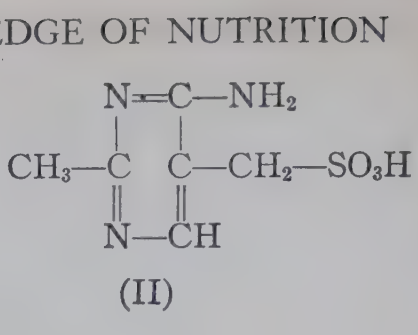
Seidell's ('17) arduous investigations, begun in 1916, were based
adsorption of the vitamin from acid solutions by means of fulle
earth. Some of his concentrates had an activity 100 times that of dri
brewer's yeast in the cure of polyneuritic pigeons. A comprehensi

ew of these and other early attempts to isolate the vitamin has
n made by Kruse and McCollum ('29).
ansen and Donath ('26), employing a modified Seidell adsorp-
method, isolated from rice polish small amounts of a highly active
rochloride. It melted at 250° and the free base had the formula
$H_{10}ON_2$. Windaus and associates ('31) employed essentially the
e procedure and obtained crystalline material with practically the
e potency but differing chemically in that the formula was
$H_{17}OH_3S$. This epochal report soon was confirmed and proof was
uced that the preparation of the Dutch workers, Jansen and
ath, also contained sulfur. However, there remained some doubt
the Dutch and German crystals were the pure vitamin. In par-
lar the bio-assays of O'Brien and Peters suggested that even con-
rates of the vitamin, prepared in Peter's laboratory, were more
nt than the crystalline materials given to them by Jansen or Win-
s. Moreover, absorption spectra of the various crystalline prepara-
s indicated lack of complete homogeneity. At this point the notable
k of Williams and coworkers ('30) began to clarify the problem.
liams et al. ('34) eventually developed a method for securing con-
nt yields of the vitamin, being able to obtain approximately 5 gm.
ton of rice polishings. This appeared to be about 25 per cent of
total amount present. The relatively large yield was due, in con-
rable measure, to the use of quinine sulfate, instead of barium
oxide, as an eluate of the activated fuller's earth.

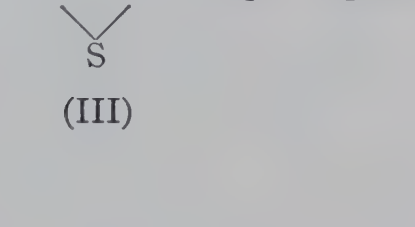
ynthesis of Thiamin.—Elucidation of the nature of thiamin and
ccomplishment of its synthesis were dependent upon the painstak-
study of minute amounts of material since only small amounts
available. By means of oxidation with nitric acid, Windaus,
esche and Grewe ('34) succeeded in a cleavage of the vitamin into
compounds. The assigned empirical formulae were: $C_7H_{11}N_3O_5$
$C_5H_5NO_2S$ (I). Shortly thereafter Williams et al. ('35) announced
mportant discovery that cleavage, without complicating reactions,
l be obtained by treatment of the vitamin with sodium sulfite
om temperature in slightly acid solution. The products were a
nic acid (II), $C_6H_9N_3O_3S$ and a basic compound, C_6H_9NOS.
latter was shown to be 4-methyl-5-hydroxyethylthiazole (Clarke
Gurin, '35). It was synthesized by Buchman ('36). Oxidation with
acid converted it into 4-methylthiazole-5-carboxylic acid, which
ntical with the substance (I) of Windaus. Potentiometric titra-
revealed that the thiazole moiety of the vitamin is present in the
of the quaternary salt. In view of this, and other important facts
cannot be enumerated here, the structural formula (IV) was
ed to the vitamin. The formulae of these products, and of the
in itself, are as follows:



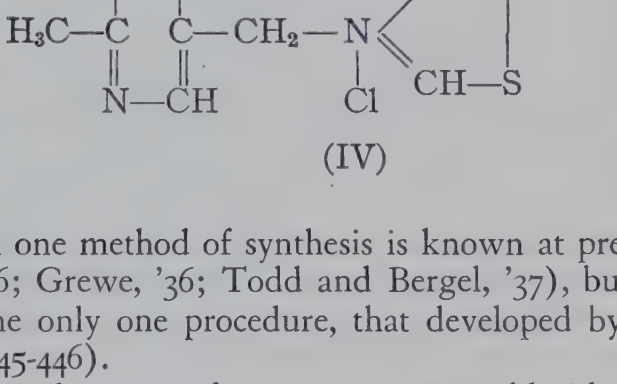
(I)



(II)



(III)

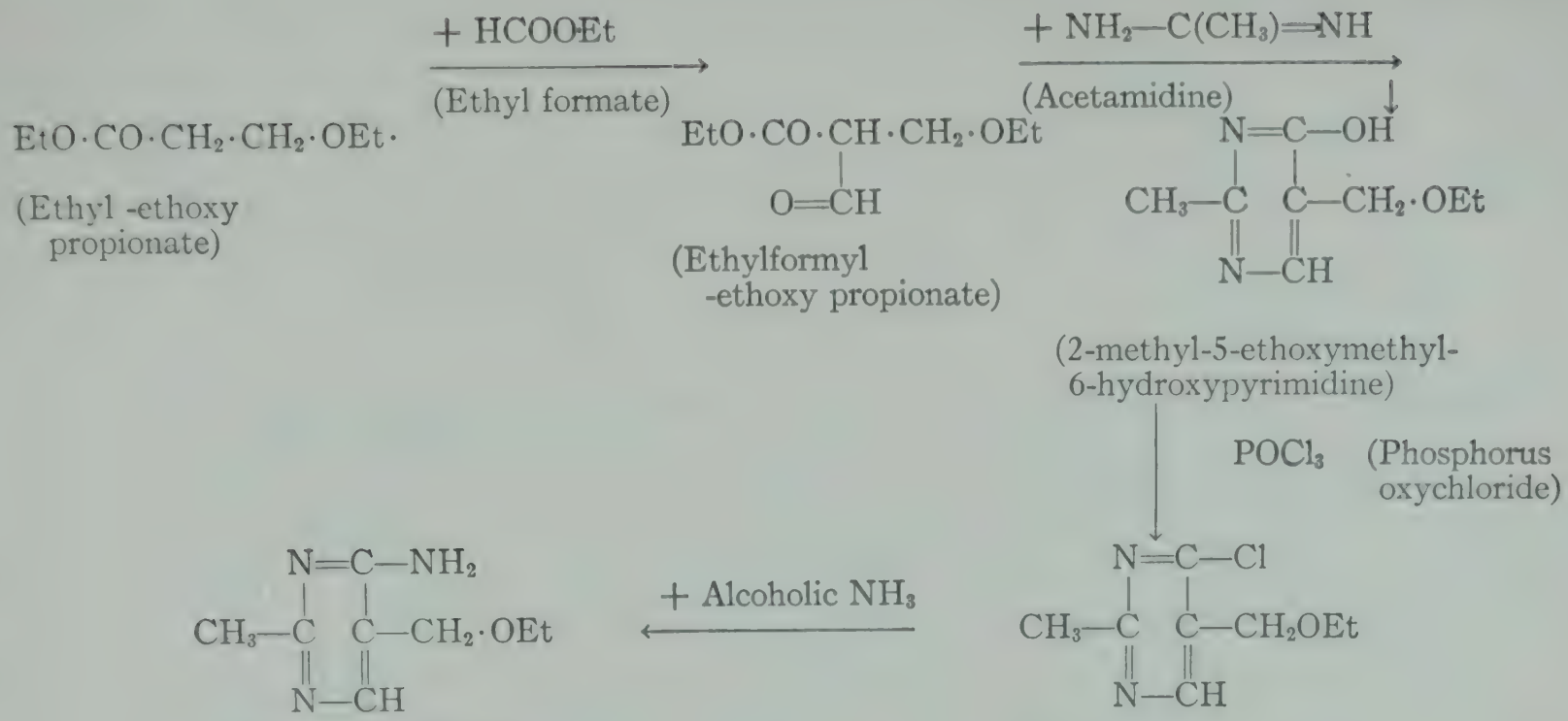


(IV)

More than one method of synthesis is known at present (William and Cline, '36; Grewe, '36; Todd and Bergel, '37), but it is sufficien here to outline only one procedure, that developed by Williams an Cline (pp. 445-446).

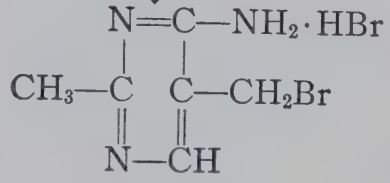
The chemical name for vitamin B_1 chloride hydrochlorid (thiamin) is 2-methyl-5-(4-methyl-5-beta-hydroxyethylthiazolium chlo ride) methyl-6-aminopyrimidine hydrochloride.

The achievement of Cline, Williams and Finkelstein, and of Clark and coworkers, in decomposing the vitamin obtained from rice polish ings into simpler cleavage products, the molecular structures of whic were known, and the subsequent building up of a molecule from rel tively simple organic compounds to form a synthetic product identica with that formed by plants in nature, furnishes an excellent illustratio of the spirit of organic chemistry. The history of chemistry is replet with comparable achievements by chemists who have taken the proc ucts of the plant and animal kingdoms, systematically decompose them into known fragments, and have then put together simple com


+ HCOOEt
(Ethyl formate)
+ NH2—C(CH3)=NH
(Acetamidine)
EtO·CO·CH2·CH2·OEt·
(Ethyl -ethoxy propionate)
EtO·CO·CH·CH2·OEt
O=CH
(Ethylformyl -ethoxy propionate)
N=C—OH
CH3—C C—CH2·OEt
N—CH
(2-methyl-5-ethoxymethyl-6-hydroxypyrimidine)
POCl3 (Phosphorus oxychloride)
N=C—Cl
CH3—C C—CH2OEt
N—CH
+ Alcoholic NH3
N=C—NH2
CH3—C C—CH2·OEt
N—CH
(2-methyl-5-ethoxymethyl-6-aminopyrimidine)

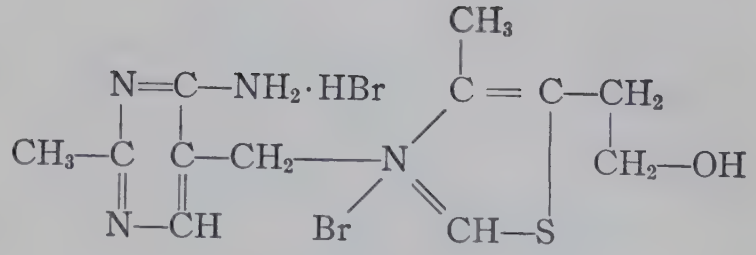
(2-methyl-5-ethoxymethyl-
6-aminopyrimidine)

HBr (Hydrobromic acid)



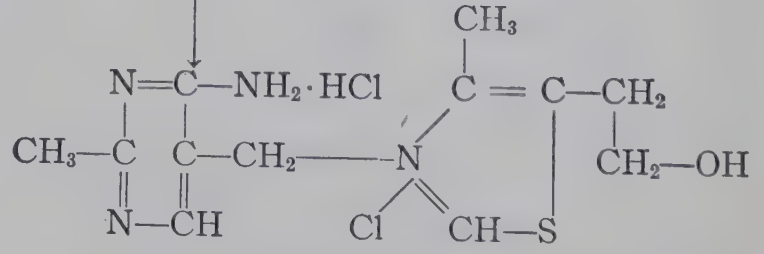
(2-methyl-5-bromomethyl-
6-aminopyrimidine hydrobromide)

4-methyl-5-β-hydroxy-
ethylthiazole



(Thiamin bromide hydrobromide)

Alcoholic
AgCl

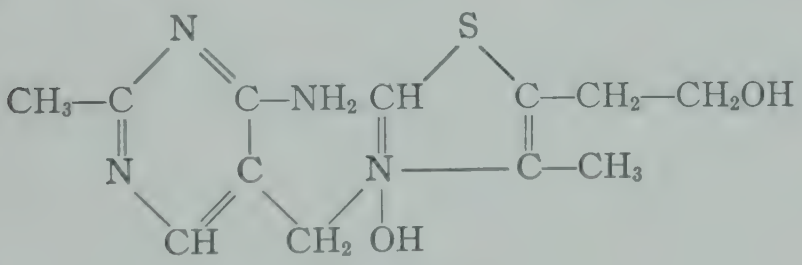


(Thiamin chloride hydrochloride)

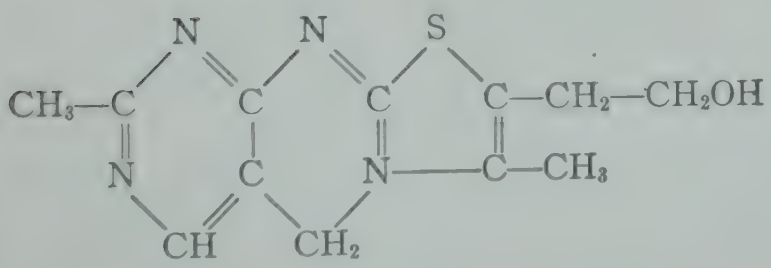
unds to build up a complex molecule identical with the natural oduct. Sugars, amino acids, polypeptides, fatty acids, fats, alkaloids, ors of flowers, respiratory pigments, synthetic rubber, etc., have all en produced in this manner in the laboratory.

Chemical Properties of Thiamin.—According to Cline, Williams d Finkelstein ('37), thiamin crystallizes from alcoholic aqueous solu- ns as the hemihydrate, $C_{12}H_{17}ON_4SCl.HCl.\frac{1}{2}\ H_2O$. It melts at 3-250°. The corresponding bromide hydrobromide forms rosettes of edles which melt at 229-231°. Two sulfates and a nitrate of the base ve been prepared by Kinnersley, O'Brien and Peters ('35). They ve shown that blue fluorescent oxidation products of pure thiamin e produced at room temperature very slowly by the action of per- anganate and manganese oxides at pH values more acid than pH . The reaction products form more rapidly about pH 7.0. The sub- nces formed are yellow in acid solution. The reactions do not split sulfur as sulfate but the state of combination of sulfur changes. is significant that the fluorescent oxidation products show biological ivity even in absence of the normal color reaction (formaldehyde- o-reaction).

Relation to Thiochrome: The above work of Kinnersley et al. is portant in relation to the studies of Kuhn, Wagner-Jauregg, van averen and Vetter ('35) who isolated from yeast a yellow basic ostance, $C_{12}H_{14}N_4OS$, which, in alkaline or neutral solutions, shows ense blue fluorescence. This substance was named thiochrome by hn et al. Its structural similarity to thiamin is indicated in the fol- wing formulae:



Thiamin (Free base)

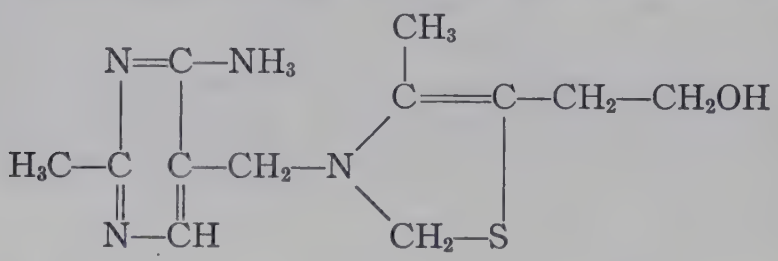


Thiochrome

That thiamin may be changed into thiochrome with relative e is indicated by the recent literature reports. Barger and cowork ('35) have formed thiochrome by the oxidation of an alkaline solut of thiamin with potassium ferricyanide. Kuhn and Vetter ('35) rep the formation of thiochrome from thiamin by means of other oxidiz agents such as hydrogen peroxide, selenium dioxide, and potassi permanganate. Also Kinnersley et al. observed the formation of sm amounts of thiochrome in alcoholic solutions of thiamin allowed stand at room temperature for several months. The reaction is acce ated in solutions approaching pH 7.

Thiamin has a pK, 4.8, corresponding to a basic group and pK about 9.0, corresponding to a pseudo-acidic or pseudo-basic group. T potentiometric behavior of thiochrome is somewhat similar, having basic pH of 5.6 and, like thiamin, a tendency for a drifting titrati value to the alkaline side of pH 7. Thiochrome has no thiamin tivity as indicated by the catatorulin test (Ogston and Peters, '36).

Reduction: Lipmann ('36) has found that thiamin is readily duced when treated with platinum black or hydrosulphite. In a pho phate solution at pH 7.5, 0.94 mol of H_2 was absorbed per mol thiamin. It is believed that the reduction product has the structure:



The mode of reaction with hydrosulphite may be regarded as indic tive of a function in the living cell, namely, that of a hydrogen a ceptor.

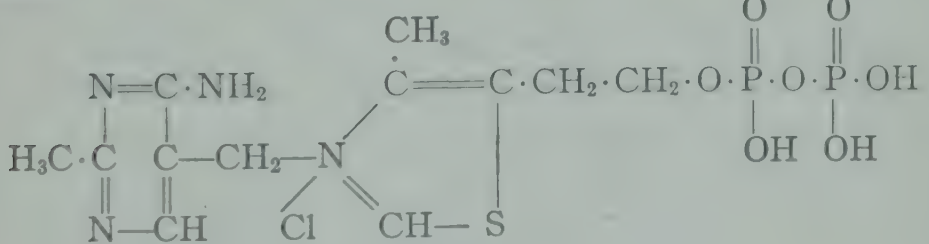
Physical Properties of Thiamin.—Wintersteiner, Williams an Ruehle ('35), among others, have studied the ultraviolet absorption c thiamin and report that two bands are present at 235 μ and 267 respectively whether alcohol or water is used as solvent. Narasimha murthy ('36), by means of electrophoresis and microcataphoresis, ha found that the isoelectric point lies between pH 9.0 and 10.0.

The reaction to heat has been the subject of inquiry almost sinc the biological discovery of thiamin. By means of heat, Smith an Hendrick, in 1926, differentiated the "vitamin B" of that period int "heat-labile" and "heat-stable" fractions. The former corresponded t the present thiamin. The vitamin, in foodstuffs, withstands ordinar cooking with practically no destruction, if the pH is not more than 7 However, several studies have shown that temperatures above 110° C.

hich may be reached in a pressure cooker, cause progressive destruc-on. As a general rule the temperature reached in the processing of anned foods causes very significant destruction of thiamin; hence nese may be regarded as of little value as a source of this substance nless actual tests prove the contrary. It should be emphasized that oods cooked or preserved in an acid medium are much more resistant o heat than those prepared in alkaline media. Various experimental ata attest to this conclusion. For example, Sherman and Burton ('26) eated samples of tomato juice during four hours at 100° C., after djusting the reaction to pH values of 4.28, 5.2, and 7.9. The thiamin estruction amounted to 20, 30, and 70 per cent respectively. Guha nd Drummond ('29) state that boiling a solution of thiamin during 4 hours at pH 1 caused no significant inactivation, but at pH 5 bout 50 per cent was inactivated. In view of the present indication nat thiamin functions in the body as a pyrophosphoric acid ester, to e discussed subsequently, it is not improbable that the stability in oods at acid reactions, is attributable to its occurrence as an ester. uch a mode of combination might cause the vitamin to be more re-stant to heat.

Miscellaneous Reactions of Thiamin.—For many years it has been nown that alkali destroys vitamin B_1 activity. Kinnersley et al. ('35, c.) have shown that hot alkali splits off sulfur as hydrogen sulfide. hey have also shown that thiamin reacts very slowly if at all with itrous acid. Morgan's ('35) observation that the thiamin activity of ulfured fruits is lowered might be correlated with the high lability of ne substance in the presence of sulfite.

Cocarboxylase.—With respect to the function of thiamin in tissue espiration it is of outstanding significance that a coenzyme, called ocarboxylase by Auhagen ('33) and required in the decarboxylation f pyruvic acid, should contain thiamin. It was isolated by Lohmann nd Schuster ('37) who showed that it is a pyrophosphoric ester of iiamin:



Cocarboxylase

The coenzyme has been synthesized by enzymatic means (Tauber, 7; Euler and Vestin, '37) and by means of phosphorus oxychloride Stern and Hofer, '37). As in the case of inorganic pyrophosphate, one

phosphate group is readily split off by means of acid hydrolysis, the other is more resistant.

The International and Other Units of Thiamin.—Before a degree of success was attained in the chemical estimation of the a neuritic vitamin, many attempts were described to estimate the s stance by means of biological tests upon pigeons and rats. Con quently, various criteria of potency have been employed and a numb of different "units" have been defined on these bases. The need uniform assay procedures, an international unit, and an internatio reference material, was met by the Second International Conferen on Vitamin Standardization, held in London in 1934. The Committ adopted as the International Standard an adsorbate prepared by Jans using the method of Seidell. A special fuller's earth is the adsorba and 3 kg. of earth contains the thiamin which is extracted from 1 kg. of rice polish by water acidified to pH 4.5 with sulfuric acid a containing 0.2 per cent of salicylic acid as a preservative against ba terial decomposition. The International Unit recommended by th Committee is the "vitamin B_1 activity of 10 milligrams of the Intern tional Standard adsorption products." In animal assays the lack of pr cision in determining the potency of any preparation containin thiamin is shown by the statement of the Committee that, "A dail dose of 10 to 20 milligrams of this (International adsorbate) prepara tion is required to maintain normal growth in a young rat on a die deficient in vitamin B_1 but complete in all other respects. The curativ day dose for a 300 g. pigeon exhibiting head retraction on a diet o polished rice is about 10 to 30 milligrams." These statements are an ex pression of the great differences in the response of individual animals some requiring two or three times as much as others to induce a com parable response. The biological assay for thiamin is, therefore, only rough approximation of the potency of a preparation. Some advanc has been made in the accuracy of reporting assays since crystallin thiamin has become available as the synthetic product. Probably poo response to a given dose in individual animals is in part to be explaine by the failure of their digestive tracts to elute the vitamin from th earth. This view is supported by the observations of Sampson an Keresztesy ('37) who found that a quinine sulfate extract of the In ternational standard earth is about twice as effective a source of th vitamin as is the earth itself. They state that rats can utilize onl about 60 per cent of the vitamin administered as fuller's earth adsorb ate.

Several attempts have been made to translate crystalline thiamin chloride hydrochloride into terms of International Units on the basi of biological tests. The following data show how great are the dis crepancies in the results:

TERNATIONAL UNITS ESTIMATED IN ONE MILLIGRAM OF CRYSTALLINE THIAMIN
(Chloride Hydrochloride)

Waterman and Ammerman ('35) (rat growth assay)	200
Moll ('35) (pigeon day dose, and rat curative method)	500
Smith and Seidell ('36) (rat curative method)	333
Kinnersley and Peters ('36) (pigeon curative method)	500
Jansen ('36) (Smith's modified curative method-rat)	300
Sampson and Keresztesy (l.c.) (curative method-rat)	200
Sampson and Keresztesy (curative method-rat-using quinine eluate)	400
Leong and Harris ('37) (Bradycardia method)	333–357

From data now available it is evident that no procedure has yet en devised for the accurate assay of thiamin in a foodstuff.

Approximate Equivalence of the Various Units.—Cowgill ('34) s carefully discussed the problem of conversion of various units. It concluded upon unequivocal evidence and logic that equivalence tween various units is, by whatever means of calculation, at best a ere approximation. Nevertheless it is possible to make rough estima- ns of the thiamin content of biological materials by means of bio- gical assay and the conversion of results from a given assay pro- dure to the unitage of another. With emphasis therefore upon these nitations it is possible to state that one gram of pure thiamin equals: 0,000 Smith curative units; 600,000 Chase-Sherman units; 300,000 oscoe units; and 300,000 International units. One International unit uller's earth adsorbate) equals 3.333 micrograms (3.333 millionths one gram) of thiamin; 20 milligram-equivalents (Cowgill); 1 Ros- e unit; 2 Chase-Sherman units; and 0.5 Smith curative unit.

Biological Tests for Thiamin.—Rats and pigeons have been gen- ally used in animal assays of biological materials. With rats the incipal criteria of potency have been those of growth and the cure polyneuritis. In practically every instance the basal diet has con- ned autoclaved yeast as a source of water-soluble vitamins other than iamin. Unfortunately the growth of animals receiving autoclaved ast plus an abundance of thiamin is not as good as in animals re- iving a similar diet with yeast which has not been autoclaved. This ggests that a basal test diet, using autoclaved yeast for the purpose ove indicated, is "incomplete" with respect to factors other than iamin. This, in addition to variability in coprophagy, genetic differ- ces in the ability to conserve thiamin in the body, variability in the ck rations employed in producing test animals, and other factors, nds to introduce considerable variability in results. Consequently, alytical data obtained by means of biological tests are, at best, only proximations of the truth.

In the case of pigeons, and other birds which have been used, the sal ration is usually polished rice. Since this food is deficient in many

respects it is apparent that results obtained thereby might be so
what variable, thus making it impossible to ascertain the exact thia
content of the assayed material.

Rat Procedures.—Two general methods are used, namely, gro
and curative. Sherman and his coworkers (Sherman and Smith,
have made elaborate studies on the estimation of thiamin in fo
by determining the smallest amount of the assay material wh
added to the basal ration, will maintain growth in young rats at
rate of 3 grams per week during 4 to 8 weeks. This unit is the Ch
Sherman unit, or the Chase unit. Chick and Roscoe ('29) modified
method in that the experimental rats, on weaning, are restricted t
thiamin-free diet until they cease to gain weight. The material to
tested is then added to the diet in graded doses in different groups
animals, and the amount which will restore growth at the rate of
to 14 grams per week is found. This is known as the Roscoe unit.
is equivalent to approximately 2 Chase-Sherman units.

In the development of a curative test, Smith ('30) modified a p
cedure introduced by Hofmeister in 1922. In this method young r
are brought into the polyneuritic state on a suitable basal diet and t
minimum dose of test material is given which will effect a cure of t
acute neuromuscular symptoms. The criterion of a definite polyne
ritic state is the tendency of the rat to develop a convulsion after bei
suspended by the tail and rotated. In this method a single dose of t
test material is given and a cure must be effected within 24 hours. Th
measures the Smith curative unit.

Pigeon Procedures.—Three general methods have been use
namely, protective, weight maintenance, and curative. The protecti
method is not at all satisfactory since it involves the determination
the amount of test material which must be added to a basal ration
polished rice in order to prevent neuromuscular symptoms. The pri
cipal objections to this procedure are inherent in the weight mai
tenance method developed by Williams and Seidell in 1916 and elab
rated by Cowgill (l.c.). Cowgill's pigeon unit, obtained by this metho
was estimated to be equivalent to 10 Chase-Sherman units. His un
is approximately equivalent to the Kinnersley and Peters' "pigeo
curative unit." The curative test of Kinnersley, Peters and Read
('28) is based upon the alleviation of acute head retraction, and oth
polyneuritic symptoms, after administration of the test material
graded doses, to pigeons made severely polyneuritic by restriction to th
thiamin-free diet. The curative unit is the weight of the curative do
divided by the number of days from cure to the return of polyneurit
symptoms. Although this procedure is recognized as superior to oth
pigeon methods, it is obviously lacking in the analytical precisio
which is desired for thiamin determinations.

The Electrocardiographic "Bradycardia" Method for Thiamin Assay.—Drury, Harris and Maudsley ('30) showed that rats in thiamin deficiency have slow heart rates and that provision of the vitamin produces rapid cures, the effect on the heart rate being proportional to the amount of the vitamin administered. The normal heart rate of the rat is 500 to 550 beats per minute. When they are restricted to a diet deficient in thiamin for about three weeks the rate falls to about 350 beats per minute. A single dose of the test material is given, and the electrocardiogram is again taken after 24 hours. The increase in rate and the time during which the increase lasts are the criteria on which the dose of the vitamin is estimated. The assay of an unknown product is carried out by testing the effect of several levels of dosage, using 4 to 6 rats, and a comparison is made simultaneously with the amount of the standard reference material necessary to produce a comparative effect. In spite of pertinent objections this procedure appears to be the most satisfactory yet devised for the assay of thiamin in foods and other materials, particularly when the concentration is very low and time is limited.

The rapidity with which thiamin affects the heart rate of deficient animals is indicated by the studies of Zoll and Weiss ('36) who showed that elevation of the heart rate and disappearance of the abnormal electrocardiographic changes occur as early as within 4 hours after administration of thiamin. Birch and Mapson ('36) have found that adenylic acid, which is associated with the nucleotides of foods, has an accelerating effect on the heart. Hence, when natural foods rich in nuclear material are assayed by this material, errors of considerable magnitude may occur. Robertson and Doyle ('37) have pointed out certain difficulties in the conduct of the bradycardia test. They could not secure consistent results.

The Catatorulin Test for Thiamin.—Peters and coworkers (O'Brien and Peters, '35) have shown that the brain of an animal deficient in thiamin has a low *in vitro* uptake of oxygen in lactate solutions. It is suggested that the vitamin acts as a coferment in its effect upon oxygen utilization by the brain. The addition of a source of thiamin accelerates oxygen uptake by brain tissue under proper conditions, and distinct effects are produced by as little as 0.1 gamma of the vitamin in 3 cc. of solution. The name of the test had its origin in the use by the investigators of the name torulin for vitamin B_1, and the observed catalytic effect described above. The results obtained by this method, when the isolated vitamin is employed, appear to be reliable, but it is not applicable to foodstuffs.

Mould Growth Method of Assay for Thiamin.—Schopfer ('35) and Schopfer and Jung ('36) state that the mould, *Phycomyces blakesleeanus*, cannot grow on a medium consisting of 10% glucose, 1%

asparagine, 0.5% $MgSO_4$, and 1.5% KH_2PO_4, but grows appreciab after the addition of 0.0005 microgram of thiamin. Within certa limits the growth is directly proportional to the concentration of t vitamin. Schopfer and Jung were able to obtain results with whe germ preparations which agree very well with those obtained by t rat growth test, but the test is not specific since autoclaved sources thiamin are active.

Fermentation Assay for Thiamin.—Schultz, Atkin and Frey ('3 find that thiamin exerts a powerful action on the rate of alcohol fermentation. In the presence of a suitable sugar-salt buffer mixture little as one gamma (0.000001 gm.) of the vitamin may be detecte They suggest that this phenomenon might be employed for thiami assay.

There are inherent difficulties in these suggested methods as a bas of quantitative assay of a foodstuff. In the assay of various kinds o foods it must be shown that absorption of essentially all of the thiami is accomplished. Especially in the case of certain vegetable foods thi question requires careful study. In respect to the methods dependen upon the preparation of thiamin concentrates it has been shown that in general, even thorough extraction with water removes far less than the total amount of the vitamin in most foods. It appears that the problem of thiamin assay of foods and biological substances cannot be adequately solved until considerably more is learned concerning the chemical combinations of this substance in such materials, and methods of quantitatively removing the vitamin therefrom.

Qualitative Tests for Thiamin.—One of the first tests with some semblance of usefulness was that described by Kinnersley and Peters ('34). Diazotized sulfanilic acid is added to an alkaline carbonate solution and, after a short interval, the unknown solution containing a trace of formaldehyde, added immediately before mixing, is added to the diazo solution. The color, which is yellow at first, but turns pink in a short time, is stable for many days. Unfortunately highly purified test solutions must be used, thus causing loss of the thiamin in purification. Acetone and reducing agents, in particular, interfere with the test.

Jansen (l.c.), on the basis of thiochrome formation when thiamin is oxidized with potassium ferricyanide, devised a procedure for estimation of the vitamin by measurement of the fluorescent thiochrome in a comparator for fluorescent solutions. Karrer and Kubli ('37) modified the procedure with a standard of comparison prepared by the oxidation of thiamin to the fluorescent thiochrome. There are inherent sources of error in these thiochrome methods which render them less reliable than the standard biological procedures. However, Westen-

ink and Goudsmit ('37) claim that they have been able to adapt the ocedure to urine analysis and that the results are satisfactory.

Another test which appears to be quite specific is that described by ebluda and McCollum ('36), who found that when a solution of ra-aminoacetanilide or methyl-para-aminoacetophenone is treated th nitrous acid and the resultant reagent is added to a solution of iamin, a characteristic purple-red compound is produced which is ble and highly insoluble in water. The compound is soluble in etone, amyl alcohol, and other such solvents and when dissolved erein can be compared in a colorimeter with known amounts of iamin similarly treated.

Melnick and Field ('38) have confirmed this test and report that e purple-red compound can be quantitatively extracted with xylene d that this extract lends itself to colorimetric evaluation. Also, they ve used permutit as a means of isolating thiamin from interfering bstances. However, this colorimetric test, like all other chemical ethods, cannot as yet be quantitatively applied to the analysis of odstuffs and biological materials owing to the lack of methods for paration of the vitamin from all interfering substances such as pro- n and some other materials.

Several reagents are known which precipitate the vitamin. Among ese are bismuth potassium iodide, reported by Naiman ('37), Rei- cke's salt, and potassium tetranitritodiaminocobaltiate (Rosenthaler,). Under suitable conditions thiamin is precipitated by picrolonic d, gold chloride, mercuric chloride, Mayer's reagent, and phospho- ngstic acid.

Thiamin in Relation to Plant Growth.—The mechanism of thia- n synthesis in, or associated with, plants has yet to be described. owever, several investigators have shown that thiamin is essential plant embryo development. It has been known for some time t tomato root tips require something for growth which yeast ex- ct supplies in addition to an otherwise suitable medium composed certain salts and sugars. Bonner ('37), and Robbins and Bartley 7) found that yeast extract can be replaced by thiamin either as pared from natural sources or as the synthetic substance. Bonner es that 0.2 gamma per cent of thiamin will replace the yeast extract imum, and 0.002 gamma has a marked influence. It is of consider- interest that Kögl and Haagen-Smit ('36) correlate the relative min content of peas with the thiamin required to produce good wth of the excised embryos. Robbins and coworkers have tested omato root tip growth the thiazole and the pyrimidine portions of min into which it is cleaved by sulfite, and from the coupling of ch the vitamin results in synthesis. They find that only the thiazole

portion is needed by the root tips. It appears, therefore, that when t thiazole is provided the tips can synthesize the pyrimidine portion the molecule, and can combine the two to produce thiamin. Th assumption is warranted on the basis of the universal presence of t complete molecule of thiamin in the growing parts of plants. Th fungus *Phycomyces* requires both the thiazole and pyrimidine inte mediates which serve it as well as does thiamin. Another fungu *Aspergillus,* grows without thiamin or either of the intermediates.

Thiamin in Relation to Bacterial Growth.—It has been know for many years that certain bacteria, particularly those normally occu ring in the digestive tracts of ruminants, are able to synthesize thiami But the precursors in such syntheses are not known. Also thiamin ac as a stimulant to the growth of some bacteria. The work of Tatun Wood and Peterson ('36) is of interest since they have secured un quivocal evidence that thiamin stimulates the growth of propionic aci bacteria. Inositol, pantothenic acid, ascorbic acid, riboflavin, nicotin acid amide, and indoleacetic acid could not replace thiamin in th function. Owing to the availability of thiamin, various compound related to this vitamin, and other important nutritive factors in pur form, it may be predicted that mechanisms of microorganism growt will be revealed in clearer perspective during the next few years.

Experiments on the Substitution of Thiamin Intermediates fo Thiamin in Pigeon Polyneuritis.—Robbins and Kavanagh ('37) stat that polyneuritic pigeons may be cured by the oral administration o sufficient amounts of both the thiazole and the brompyrimidine inte mediates. These results seem to indicate that thiamin may be syn thesized from its intermediates in the crop or in the tissues of th pigeon. Williams stated that, theoretically, one would suspect such combination of the pyrimidine and the thiazole to occur, though per haps at a very slow rate unless catalyzed.

REFERENCES

References to the literature cited in the introduction will be found i Chapter II.

American Medical Association, Council on Pharmacy and Chemistry (1937). J. Am. Med. Assoc., *109,* 952.

Funk (1912). J. State Med., *20,* 341.

Vedder and Williams (1913). Philippine J. Sci., Sect. B., *8,* 175.

Seidell (1917). J. Biol. Chem., *29,* 145; ibid. (1926), *67,* 593; J. Indust Eng. Chem. (1921), *13,* 1111; J. Am. Chem. Soc. (1922), *44,* 2042.

Kruse and McCollum (1929). Physiol. Rev., *9,* 126.

Jansen and Donath (1926). Proc. Kon. Akad. Wetensch., Amsterdam, *29,* 1390.

INDAUS, TSCHESCHE, RUHKOPF, LAQUER and SCHULTZ (1931). Nachr. Ges. Wiss., Göttingen, III, *207;* idem, Ztschr. physiol. Chem. (1932), *204,* 123.
ILLIAMS, WATERMAN and GURIN (1939). J. Biol. Chem., *87,* 559.
ILLIAMS, WATERMAN and KERESZTESY (1934). J. Am. Chem. Soc., *56,* 1187.
INDAUS, TSCHESCHE and GREWE (1934). Ztschr. f. physiol. Chem., *228,* 27.
ILLIAMS, WATERMAN, KERESZTESY and BUCKMAN (1935). J. Am. Chem. Soc., *57,* 536.
ARKE and GURIN (1935). J. Am. Chem. Soc., *57,* 1876.
CHMAN (1936). J. Am. Chem. Soc., *58,* 1803.
ILLIAMS and CLINE (1936). J. Am. Chem. Soc., *58,* 1504.
REWE (1936). Ztschr. f. physiol. Chem., *242,* 89.
ODD and BERGEL (1937). J. Chem. Soc., 364.
LINE, WILLIAMS and FINKELSTEIN (1937). J. Am. Chem. Soc., *59,* 1052.
NNERSLEY, O'BRIEN and PETERS (1935). Biochem. J., *29,* 2369.
CHN, WAGNER-JAUREGG, VAN KLAVEREN and VETTER (1935). Ztschr. f. Physiol. Chem., *234,* 196.
RGER, BERGEL and TODD (1935). Nature, *136,* 259.
UHN and VETTER (1935). Ber., *68B,* 2375.
STON and PETERS (1936). Biochem. J., *30,* 736.
PMANN (1936). Nature, *138,* 1097.
INTERSTEINER, WILLIAMS and RUEHLE (1935). J. Am. Chem. Soc., *57,* 517.
ARASIMHAMURTHY (1936). Indian J. Med. Research., *24,* 221.
ERMAN and BURTON (1926). J. Biol. Chem., *70,* 639.
CHA and DRUMMOND (1929). Biochem. J., *23,* 880.
ORGAN (1935). Am. J. Pub. Health, *25,* 328.
CHAGEN (1933). Biochem. Ztschr., *258,* 330.
HMANN and SCHUSTER (1937). Naturwissenschaften, *25,* 26.
UBER (1937). Science, *86,* 180.
LER and VESTIN (1937). Naturwissenschaften, *25,* 416.
ERN and HOFER (1937). Science, *85,* 483.
MPSON and KERESZTESY (1937). Proc. Soc. Exper. Biol. & Med., *36,* 30; *37,* 686.
ATERMAN and AMMERMAN (1935). J. Nutr., *10,* 35.
OLL (1935). E. Merck Jahresbericht., *49,* 56.
ITH and SEIDELL (1936). U. S. Pub. Health Reports, *51,* 685.
NNERSLEY and PETERS (1936). Biochem. J., *30,* 985.
SEN (1936). Ztschr. f. Vitaminforsch., *5,* 254.
ONG and HARRIS (1937). Biochem. J., *31,* 672.
WGILL (1934). "The Vitamin B Requirement of Man." Yale U. Press. New Haven.
ERMAN and SMITH (1931). "The Vitamins." Chemical Catalog Co., New York.
ICK and ROSCOE (1929). Biochem. J., *23,* 498.
ITH (1930). U. S. Pub. Health Reports, *24,* 116.

Kinnersley, Peters and Reader (1928). Biochem. J., *22*, 276.
Drury, Harris and Maudsley (1930). Biochem. J., *24*, 1632.
Zoll and Weiss (1936). Proc. Soc. Exper. Biol. & Med., *35*, 259.
Birch and Mapson (1936). Nature, *138*, 27.
Robertson and Doyle (1937). Proc. Soc. Exper. Biol. & Med., *37*, 139
O'Brien and Peters (1935). J. Physiol., *85*, 454.
Schopfer (1935). Ztschr. f. Vitaminforsch., *4*, 67, 187.
Schopfer and Jung (1936). Compt. rend. Soc. de biol., *122*, 249.
Schultz, Atkin and Frey (1937). J. Am. Chem. Soc., *59*, 9168.
Kinnersley and Peters (1934). Biochem. J., *28*, 667.
Karrer and Kubli (1937). Helv. Chim. Acta, *20*, 369.
Westenbrink and Goudsmit (1937). Rec. trav. chim., *56*, 803.
Prebluda and McCollum (1936). Science, *84*, 488.
Melnick and Field (1938). Proc. Am. Soc. Biol. Chem., 32nd Annu
Meeting, Baltimore.
Naiman (1937). Science, *85*, 290.
Rosenthaler (1936). Ztschr. f. Vitaminforsch., *5*, 276.
Bonner (1937). Science, *85*, 183.
Robbins and Bartley (1937). Science, *85*, 246; idem. Proc. Nat'l Aca
Sci., U.S.A. (1937), *23*, 385.
Kögl and Haagen-Smit (1936). Ztschr. f. physiol. Chem., *243*, 209.
Tatum, Wood and Peterson (1936). Biochem. J., *30*, 1898.
Robbins and Kavanagh (1937). Proc. Nat'l Acad. Sci., U.S.A., *23*, 499.

CHAPTER

XIX

Nutritional Significance of Thiamin (Vitamin B_1)

HE EARLIER observations on beriberi and on its analogue, polyritis in fowls, pigeons, dogs, and rats, have been excellently sumrized by Vedder ('13) and by Funk ('22). Scheube (1881) and lz ('03) demonstrated the existence of polyneuritis in beriberi, and eved that the central nervous system was unaffected. Their studies to the general belief that the disease was a peripheral neuritis. e cardinal symptoms of beriberi are those of peripheral neuritis, diac insufficiency, and a generalized tendency to edema. Of the two ms of the disease, the wet and the dry beriberi, it now appears that former, characterized by edema, represents the effects of protein ciency superimposed upon vitamin deficiency. Vedder suggested the enlargement of the heart in beriberi is the sequel to weakening nuscle fibers and consequent hypertrophy to meet the necessity of ulation. Also, the relationship between thiamin and carbohydrate abolism was noted early in the history of beriberi. In 1914 Funk erved that polyneuritis follows more rapidly if the carbohydrate l is increased in diets deficient in the antiberiberi factor. Subsent researches have demonstrated a very definite relationship been thiamin and carbohydrate metabolism. This will be discussed he present chapter in addition to various other nutritional aspects hiamin which include the pathologic effects of thiamin deficiency, age and excretion, requirements of various age groups, the extent hiamin deficiency in human populations, and therapeutic uses of min.

The Principal Effects of Thiamin Deficiency.—As pointed out e, the cardinal symptoms of thiamin deficiency are degeneration he nervous system, cardiac enlargement and dysfunction, edema, ointestinal disturbances, muscular atrophy, and anorexia. A de-

tailed account of the pathologic and physiologic changes which c acterize these general symptoms, in variable degree, would no profitable here since we are concerned only with those general ef of thiamin deficiency which suggest, with scant equivocation, the f tional nature of this nutrient. The rationality of this point of vie emphasized by Wolbach ('37), whose pathologic studies of vita deficiencies have contributed in an important way to our unders ing of the roles of vitamins in the body.

Nervous system: Before polyneuritis was produced experimen in animals by depriving them of thiamin, beriberi was recognized disease involving the nervous system. But even today it is not yet c whether the neuritic symptoms are directly attributable to lesion the nerves or functional interference owing, perhaps, to the accum tion of metabolic products, particularly pyruvic acid and perhaps la acid.

Some of the nervous symptoms of thiamin deficiency in hum are numbness and tingling sensations, particularly affecting the fin and usually associated with a gnawing pain which runs up the a especially at night. The tingling sensation is of the "pins and need type. Usually one side of the body is more affected than the ot There are cases, however, in which cardiac dilation and sudden de occur before evidences of nerve dysfunction are manifested.

In birds and rats marked symptoms of lesions in the nervous s tem appear soon after restriction of such animals to a thiamin-defici diet. Sherman and Elvehjem ('36a) produced polyneuritis in you chicks after two weeks on the experimental ration. In rats (Davis and Stone, '37) thiamin deficiency is marked by a paralysis of extremities, equilibratory disturbances, priapism, convulsions, and to retractions of the head. Pathologically, the outstanding effect is that disintegration of the myelin sheaths of the peripheral nerves and vac lation and liquefaction necrosis of the ganglion cells of the mes cephalon, metencephalon, and anterior horns of the spinal cord. Zi merman and Burack ('34) believe that disintegration of myelin shea is not primarily related to the clinical symptoms. In support of t view is the fact that rats on thiamin-deficient diets may die witho developing nervous lesions.

As shown by Davison and Stone, among others, inanition alo may cause as marked change in the peripheral nerves as thiamin d ficiency. Moreover, the lesions are essentially similar in both instanc Finally, as shown by Wolbach (l.c.), no differences could be found the nerve lesions present in pigeons allowed to succumb to po neuritis and those in which functional recovery had been induced thiamin administration. One can conclude, therefore, with Wolba

at, "On the whole, it seems best to regard the primary pathologic ects of vitamin B_1 (thiamin) deficiency as not demonstrable at pres- t and to regard all the pathologic changes thus far recorded, includ- g the myelin sheath lesions, as secondary effects."

Cardiac enlargement and dysfunction: The cardiac enlargement nerally characteristic of human beriberi was ascribed by Aalsmeer d Wenckebach ('29) to edema of the cardiac musculature, but New- mb ('30) was unable to note any significant difference in the water ntent of cardiac muscle from cases of human beriberi and pigeon lyneuritis. Thus the latter worker concluded that cardiac enlarge- ent in the former case is a true hypertrophy.

More recently Wenckebach ('34) has described the beriberi heart as aracterized by enlargement with rapid rate and a throbbing systolic pulse, large bounding pulsations in the peripheral arteries, venous gorgement, warm extremities and edema. As shown by Weiss and ilkins ('36) the electrocardiograms in such cases are abnormal, being aracterized by changes in the T waves, low amplitude, and prolonga- n of the electrical systole (Q-T). Birch and Mapson ('36) believe at observed bradycardia is due to a failure in the deaminase mechan- n, resulting in increased accumulation of adenylic acid in the tissue.

The relationship between cardiac changes and the composition of e blood is indicated in the above, since there is a tendency toward v plasma protein values, hyperglycemia, increased pyruvic acid, and ssibly increased lactic acid. These blood changes are suggestive of lure in the metabolism of carbohydrates, as subsequent discussion ll definitely verify, and in view of this it is easy to conclude that the art does not function normally owing to the interference with car- hydrate metabolism in that organ. The changes in heart rate and ctrocardiographic behavior have already been discussed in relation thiamin estimations (Chapter XVIII).

Edema: Human beriberi is frequently accompanied by edema. In probability dietary deficiencies other than thiamin are related to condition, as emphasized by Youmans ('36) and discussed by lder and Wilbur ('37). Probably protein inadequacy is of consider- e importance.

Gastrointestinal dysfunctions: Gastrointestinal disturbances are quent accompaniments of human beriberi but perhaps more attri- able to other factors such as intestinal parasites, dysentery, malaria, than to a direct deficiency of thiamin. Cowgill ('34) has ade- tely discussed this aspect of beriberi and he points out that condi- s such as amebic dysentery, commonly found in beriberi locali- may constitute the precipitating cause of the deficiency disease. convincing argument is that beriberians, in many instances, might

have sufficient amounts of thiamin in the diet, although at a criti low level, to prevent the disease if they were not afflicted with diar and attacks of fever. The latter increases the tissue requirements thiamin and the former causes an inefficient utilization of the vita owing to its loss in the watery stools.

In experimental thiamin deficiency the complicating factors of above nature are largely ruled out and here also dysfunction lesions of the gastrointestinal tract are said to be found. Cowgill e ('26) noted loss of tone in the gastric musculature of thiamin-defic dogs. This is accompanied by gastric hypochlorhydria, according Cowgill and Gilman ('34), who believe that the characteristic anor is related to this condition. Sparks and Collins ('35) have repeated substance the studies of Hargreaves, Fletcher and Dickson ('31) have confirmed the latter in the conclusion that thiamin deficiency rats causes a marked increase in the volume of the colon. This dem strates that thiamin has some role, either directly or indirectly, in maintenance of intestinal tone. The claims of several workers, incl ing Eddy and Dalldorf ('37), to the effect that thiamin deficie causes erosions and ulcers of the stomach, are not substantiated Simpson ('36). The latter's studies are convincing and at least t place doubt on the occurrence of gastric lesions under such diet conditions.

The evidence at present is only presumptive concerning thiamin human gastrointestinal dysfunctions. Vorhaus, Williams and Wat man ('35) noted beneficial results when 8 cases of gastrointesti hypotonia were treated with 10 mg. daily doses of thiamin given mouth.

Without citing further from the numerous reports on this subje it may be stated that the gastrointestinal changes associated w thiamin deficiency have not been completely demonstrated as havi direct origin in the deficiency of this vitamin. Nearly all the stud thus far reported have been complicated by inadequacy of oth nutritive factors and in practically all instances the sources of thiam were concentrates or merely yeast, wheat germ, or other such materi containing relatively large amounts of this vitamin. In conclusi therefore, thiamin deficiency undoubtedly affects the gastrointesti tract, causing considerable anatomical and functional changes, b further investigation is needed, using improved test diets and pu thiamin, in order to clarify the problem.

Muscular atrophy: Although muscular atrophy is a prominent, b nonspecific, symptom of thiamin deficiency, scarcely anything wor while can be recorded about it. Since anorexia and resultant inaniti are marked, it may be assumed that the fall of body weight a muscular wasting are attributable to these factors.

Anorexia: Loss of appetite is one of the first observable symptoms thiamin inadequacy. This occurs in various other deficiency diseases t in less degree. In fact, we do not know of any dietary inadequacy hich occasions such a marked degree of anorexia. As pointed out by erman ('34), the role of this vitamin certainly is not that of making e food appetizing ". . . for when the vitamin is given separately e experimental animal will return with appetite to the same food hich it had previously refused." That the mediation of thiamin in e mechanism of appetite is not through repair of structural injuries, used by deficiency of the vitamin, is indicated by the dramatic res- ration of desire for food when thiamin is administered (Cowgill,). This may occur in 24 hours. Some suggestion of the general ationship between thiamin and appetite is indicated by the signifi- nt correspondence between the caloric needs of the body and desire food. In thiamin deficiency carbohydrate metabolism appears to be entially blocked owing to failure in the breakdown of pyruvic and tic acids. It appears that appetite for food is influenced in some man- r by the blood level of intermediates of carbohydrate metabolism. herefore, it may be postulated, an interference with this essential ocess, as occurs in thiamin deficiency, causes a loss of appetite. This, course, is mere speculation but it should be suggestive of the scope an important problem in nutrition, namely, the nature of appetite.

Other effects of thiamin deficiency: There is lack of clear-cut evi- nce concerning thiamin in relation to the sexual status of animals d man. Evans' ('28) work suggests that sex interest is decreased in absence of this vitamin, but there is no indication in his results t the anatomical and functional integrity of at least the male re- oductive system is directly affected. Evans and Burr ('28) report crease in lactating ability and suppression of ovulation has been ed in rats by Evans and Bishop ('22).

Wolbach (l.c) has discussed the enlargement of the islands of ngerhans, noted by several workers, and relates it to the disturb- e in carbohydrate metabolism. McCarrison ('19) observed that the enals hypertrophy. This may be of significance in relation to car- ydrate metabolism and sexual physiology. However, in most of se studies it is possible, if not probable, that complicating factors erfered owing to the lack of pure thiamin.

Blood changes are especially significant, since as subsequent dis- sion will reveal, they suggest unmistakably the general nature of amin action in the body. The most important of these appear to be increase in glucose and pyruvic acid.

Effects of Thiamin in Massive Amounts.—It was not possible to ke conclusive determinations of the effects of thiamin in massive ounts until this vitamin became available in pure form. Since that

time several valuable investigations have been made with the re
that practically all the evidence demonstrates complete non-toxicity
all practical uses of the vitamin. Vorhaus, Williams and Waterm
(l.c.) reported that the oral ingestion by man of single doses
thiamin up to 90 mg. (27,000 I. U.) were without the slightest unfa
able effects. Weiss and Wilkins ('37) were not able to note harm
effects although they administered average doses of 50 mg. daily a
at times as much as 130 mg. daily. Hecht and Weese ('37), on the b
of studies on the cumulative effect of repeated massive doses
thiamin, concluded that there is no danger from prolonged ingest
of thiamin unless the dosage is exceedingly high, perhaps about 7
times a normal dose of 0.08 mg. of thiamin per kg. of body weight.

Physiological Role of Thiamin.—An analysis of all the evidence
present suggests that thiamin is definitely concerned with tissue
piration, particularly as it relates to the metabolism of pyruvic a
It demonstrates that there was justification in the early suggestions t
disorders observed in polyneuritis may be due to an accumulation
intermediate substances arising from carbohydrate degradation, wh
cannot be metabolized in the absence of thiamin. Especially signific
now is the early observation of Funk ('14) that an increase in
carbohydrate ingestion causes a more rapid production of polyn
ritis in pigeons on a thiamin-deficient ration. Another significant
vance was the finding of Collazo ('23) that the introduction of c
siderable amounts of carbohydrate into the crops of normal a
thiamin-deficient pigeons invariably caused death in the latter but
in the former, and it was shown by Rubino and Collazo ('23) that
carbohydrate thus introduced was actually absorbed.

The more recent studies have shown that thiamin deficiency
accompanied by a progressive diminution in carbohydrate toleran
(Kauffmann-Cosla, et al., '32). Lewinson ('37) noted that thiam
deficiency causes a derangement of carbohydrate metabolism whi
precedes the nervous disturbances and is manifested as hyperglycem
Also, it was shown by Roche ('31) that in polyneuritis there is
accumulation in the blood of intermediate products of carbohydr
metabolism and an increase in the C:N ratio in the urine. On the o
hand this derangement of carbohydrate metabolism has been attribut
to reduction in the amount of oxidizing enzymes, or to interferen
with their activity; on the other, it has been attributed to inaniti
The latter view was championed by Drummond and Marrian ('2
who insisted that the rapid diminution in body weight, the fall of bo
temperature, and the hyperglycemia are the result of inanition rath
than of direct deficiency of the vitamin. In accordance with the mo
recent findings, particularly those of Lohmann and Schuster ('3
this view is not easily reconciled with the evidence. Lohmann a

huster made the important observation that a pyrophosphate of amin isolated from yeast is cocarboxylase. Since this coferment creases the *in vitro* oxygen uptake of brain tissue from thiamin-ficient animals it is indicated that the vitamin does play an active e in tissue oxidations, and that hyperglycemia, loss of weight, etc., directly attributable to a deficiency of the vitamin in the tissues. A full discussion of this significant subject would exceed the limitations of space in this chapter. It suffices to briefly mention the important contributions of Peters ('36) and coworkers at Oxford University, whose persistence in investigations of thiamin action has added eatly to an understanding of this problem. They have unequivocally monstrated that thiamin is a catalyst in the oxidative removal of lower degradation products of glucose metabolism. At first the xford workers (Kinnersley and Peters, '29) favored the lactate dase theory of thiamin action, basing their conviction, in part, on an apparent increase in the amount of lactic acid in the brain of geons deprived of thiamin. Later a test for pyruvic acid was found eters and Thompson, '34) and it was demonstrated that pyruvic d accumulates in the blood of thiamin-deficient pigeons (Johnson,). More recently Peters (l.c.) has discarded the lactate oxidase ory and now concludes that thiamin ". . . is related specifically pyruvate oxidase in its aerobic reaction." This specific action nifests itself in two ways, (a) by increasing the oxygen uptake der precisely defined conditions, the so-called catatorulin effect, and by decreasing markedly the pyruvate formed by thiamin-icient brain tissue.

Sherman and Elvehjem ('36b), by means of a thiamin-deficient t which produces polyneuritis within two weeks in chicks, further cidated the subject when they showed that heart and kidney tissue s similarly to brain tissue, with respect to pyruvic acid. Moreover, y have shown that the removal of pyruvate injected intravenously polyneuritic chicks is much slower than in normal chicks. These a further suggest that in thiamin deficiency there is a failure in tissue metabolism of pyruvic acid. The findings correlate beauti-y with the demonstration of Lohmann and Schuster that a pyro-sphate of thiamin is cocarboxylase. Since this coferment has the torulin effect it strengthens the view that thiamin is indispensable he metabolism of carbohydrate.

Finally, it is of importance to note that methyl glyoxal, the aldehyde pyruvic acid, has been found in the blood and urine of beriberians animals on a thiamin-deficient diet (Arakawa, '30; Chiba, '32; a, '35). Geiger and Rosenberg ('33) noted this carbohydrate de-dation product in infants with toxic symptoms. It has been gested by Platt and Lu ('36) that a determination of bisulfite-

binding substances (B.B.S.) such as methyl glyoxal and pyruvic a in blood and urine may be used as a criterion in the diagnosis thiamin deficiency. This is plausible but, as shown by Wilkins, Ta and Weiss ('37), an increase in B.B.S. of the blood occurs in conditi probably unrelated to thiamin deficiency. It would seem that a pron ing procedure in the determination of mild degrees of deficiency the estimation of B.B.S. before and after the adminstration o standard dose of thiamin. Any decrease in the level, after thiam dosage, might plausibly be attributed to an inadequacy of thiam The extent of this diminution would measure the degree of deficien

State of Cocarboxylase in Tissues.—Since the very recent covery that cocarboxylase, essential in the decarboxylation of pyru acid, is the pyrophosphate of thiamin, a precise consideration thiamin in carbohydrate metabolism must be in terms of its relat to cocarboxylase. The function of cocarboxylase in yeast fermentat is clear but its role in animal metabolism has not been satisfactor elucidated. A question of prime importance is the form in wh thiamin occurs in plant and animal tissues since this is significant respects its availability in metabolic processes as well as its mode action. Tauber ('37) has claimed that much of the thiamin in pla is unphosphorylated. According to Lipmann ('37) all of the thiam in mammalian tissues is in the form of cocarboxylase. But the evider in both instances is only indirect. Tauber ('38) has adduced evider that phosphorylation of thiamin, i. e., formation of cocarboxyla occurs in the presence of a duodenal enzyme preparation. The enzym system cannot hydrolyze the coenzyme. Kidney tissue, according Tauber, rapidly hydrolyzes cocarboxylase. The studies show th coenzyme is dephosphorylated by the kidneys before being excret in the urine. Lipschitz, Potter and Elvehjem ('38) believe that thiam and cocarboxylase exist in dynamic equilibrium *in vivo*. If thiam must be phosphorylated before it can participate in metabolic proces it is conceivable that under some conditions phosphorylation is i hibited and, as a consequence, thiamin is not utilized. As shown Lipschitz et al. (l.c.), phosphorylation is inhibited by iodoacetic ac The action of cocarboxylase is not affected by iodoacetic acid.

Storage and Excretion.—The investigations previous to 1923 we inconclusive regarding storage and excretion of thiamin. In 19 Osborne and Mendel found that the content of thiamin in the live of rats is greatly reduced when the animals are deprived of th vitamin. Thus it was early learned that tissues must be constant supplied with thiamin if they are to contain it even in modera amounts. An indication of the rapid loss of the vitamin, when it not replenished by the diet, is given in the work of Westenbrink ('3 who found that in rats the heart, liver, kidneys, muscle, lung, splee

omach, and intestine become thiamin-depleted after only 5 weeks n the deficient diet. And, as claimed by Graham and Griffith ('32), ne rapid loss of thiamin, under such dietary conditions, has no nalogue with respect to components of the vitamin B-complex, ssayed as a whole.

Leong ('37), using the bradycardia method of assay, has reported alance studies in which the test animals, adult rats, were given doses f thiamin ranging from 0 to about 700 I. U. per rat daily. The uantity in the feces was 0.5 to 1.5 I. U. per day, depending upon ne bulk of the feces. But the urinary excretion became larger in roportion as the intake was increased. When scarcely any thiamin as given it amounted to 0.1-0.4 I. U. daily, a fraction of this being erived from the tissue stores. When the intake ranged from 7.5 to I. U. daily, the urinary output increased from 0.6 to 12.8 I. U. hen 250 I. U. were injected subcutaneously the amount excreted as about 75 per cent of the intake. A large percentage of this was the urine. But with oral ingestions of 100 to 700 I. U. daily, about I. U. were unaccounted for in the excreta, thus indicating the proximate amount of destruction in the body. Leong estimates, owever, that in cases of thiamin deficiency of the diet a greater ercentage of the vitamin withdrawn from the tissues is destroyed.

The significance of these data is indicated in relation to the utiliza- on and retention of thiamin in man. For example, Knott ('36), on e basis of 23 balance studies conducted with 8 children from 4 to years of age, concluded that the body is not capable of building a significant reserve of thiamin, owing to the rapid depletion which curs on low ingestion levels. The results indicate a definite trend ward higher retentions of thiamin when the level of intake is latively high. The levels which resulted in highest retentions were und to be about 27 I. U. per kilogram of body weight. These dings are supported by those of Roscoe ('36) and Harris and Leong 36). The former worker, using the rat curative test, could not find ore than traces of the vitamin in urine from normal adults receiving hospital diet. The addition of 720 I. U. of thiamin daily to this et was followed by the daily excretion of 167 to 333 I. U. in the ine. It seems probable that a considerable amount of the remainder as retained, owing to inadequacy of the hospital diet in meeting e tissue needs for the vitamin. Harris and Leong, in their search r a simple method of estimating the state of thiamin nutrition in man subjects, also found a marked correspondence between the nount of thiamin in the diet and the level of excretion in the urine.

Undoubtedly the next few years will witness notable advances in owledge of the storage and excretion of thiamin, since data of such ture are essential to an understanding of human and animal re-

quirements for the vitamin. We may safely conclude at present th appreciable storage of thiamin within the body has not been demo strated and it probably cannot occur. This suggests the need adequate amounts of the vitamin in the diet at all times.

Factors Affecting the Requirements for Thiamin.—At least th important factors may operate in determining the amount of thiam that should be in the diet in order to satisfy the body's need for t vitamin. They are (a) the absorptive status of the gastrointesti tract, (b) the degree of thyroid activity and (c) the amount a quality of the fat in the diet.

Scarcely anything can be recorded at present concerning absorpti of thiamin since there is a paucity of clear-cut experimental wo on this subject. Instances are on record, however, where parente dosage was more effective than oral ingestion of the vitamin. Examp of poor absorption are conditions in which food is not well digeste or in which there is diarrhea or inflammatory diseases of the stoma or intestines. An adequate discussion of the problem is given Cowgill ('34, l.c.).

A considerable amount of data is accumulating regarding thyro activity and thiamin. This is briefly reviewed by Carpenter and Shar less ('37), whose studies suggest that some factor present in yea which is lost when the yeast is autoclaved and which is not supplie by a vitamin B-containing extract, causes an increase in the iodi content and concentration of the thyroid.

Sure and Buchanan ('37) have published results which show th in experimental hyperthyroidism protection can be afforded by inge tion of large amounts of pure thiamin. On the basis of these relatio ships Sure and Smith ('34) suggested that thiamin might be use in the treatment of toxic goiter, particularly in non-operative case There is considerable experimental basis for this view, particular the thorough work of Cowgill and associates (Cowgill, '34, l.c which unequivocally demonstrates a high correlation between th total energy metabolism and the thiamin intake necessary to maintai good health.

Probably of relatively less practical importance is the so-calle sparing action of fats on thiamin. Several years ago Evans and Le kovsky ('29, '31, '32, '35) found that liberal inclusion of fat in th diet will enable an animal to withstand for many weeks the wit drawal or omission of thiamin from the diet and that in the presen of fat better growth will occur at any given level of thiamin ingestio than in the case with a fat-free diet. It was found that myristin an caprylin are more effective than others, and that natural fats a superior to any of the single glycerides. Tristearin exerted no sparin action; this they suggested was due to its poor absorption, its bu

the food causing partial starvation. Substantiation of their general ndings has been published by several workers including Salmon nd Guerrant ('30) and Whipple and Church ('36). But Kemmerer nd Steenbock ('33), Sure and Buchanan ('35), Westenbrink ('35) nd others have reported conflicting or contradictory results. Recently almon and Goodman ('37) have claimed that the effectiveness of ters of single fatty acids in alleviating the symptoms of thiamin eficiency in rats depends upon the length of the carbon chain of e fatty acid. The effectiveness was maximum at the 8-carbon acid nd decreased in each direction from this point.

The significance of these data as it concerns the mechanism of iamin action is, as yet, obscure. But the evidence that the vitamin ts as an essential factor in carbohydrate metabolism, at the pyruvic id stage, raises the question as to the role which it may play in e synthesis of fat. Whipple and Church have shown that the main ctor in the weight increase, following the administration of thiamin deficient animals, is the accumulation of fats which, in their periments, apparently could only be formed from carbohydrate. oreover, the synthesis of fats by rats during response to thiamin erapy was indicated by the high respiratory quotient of such animals. cHenry ('37) has confirmed this observation. It is indicated, there-re, that in the absence of thiamin pyruvic acid accumulates, whereas the presence of the vitamin fat is formed, presumably with pyruvic id as an intermediary substance. The latter is supported by the scovery of Krebs and Johnson ('37) that hydroxybutyric acid can formed from pyruvic acid by the tissues. On the basis of these servations it is at least very interesting that the thiamin content pork is relatively much higher than in beef. The hog is exception-y able in the conversion of carbohydrate into fat.

Other factors affecting the requirements for thiamin might be scussed but it suffices to mention the work on riboflavin as a sparer the vitamin. Evans, Lepkovsky and Murphy ('34), as well as hers, had noted that autoclaved yeast, presumably without thiamin tivity, appeared to spare thiamin in the rat. Ellis and Zmachinsky 37) studied the question and found that growth and length of rvival in rats deprived of thiamin could be increased by supple-entation with riboflavin.

Thiamin Requirements of the Body.—Owing to the apparent k of ability for storage of thiamin in the body, and in view of rapid loss from the tissues during short periods of deficiency, it ould seem that optimum nutrition, with respect to this factor, nnot be maintained unless the diet regularly contains it in adequate nounts. At present, however, although there are many published imates of requirements for various bodily functions, based in only

a few instances upon carefully controlled and dependable procedu there is no final basis for opinion as to the thiamin requirem for any bodily function. Without the assemblage of lengthy ar ments in support of this view, one has only to cite the fact that method is yet known for the reasonably accurate estimation border-line degrees of thiamin deficiency. Without even a good terion for such a status it is presumptious to discuss with certai thiamin requirements in terms of absolute units. But it is import to utilize such information as is available for it is useful and depe able for very rough estimations.

The present knowledge is based in considerable measure up Cowgill's ('34, l.c.) comprehensive investigations of many types diets. In general he has found that the minimum thiamin requi ments tend to vary greatly between individuals, and in the sa individual under different physiological conditions. Cowgill's attem to express mathematically the thiamin requirement has resulted the proposal of a formula which embodies the body weight and ener requirement as variables. Probably the formula anticipates importa information which is not yet established but as an approximation the general truth it does appear to be of some value. It is:

Thiamin requirements = Body weight + Caloric requirement a constant.

Possibly important practical factors which are not represented in t formula are: (a) ratio of carbohydrate in the diet to fat and protei (b) water excretion, (c) state of thiamin in the food, and (c absorptive capacity of the gastrointestinal tract.

It is of some interest and value to cite certain opinions regardi thiamin needs. The Council on Pharmacy and Chemistry of t American Medical Association ('36) concluded, on the basis of know edge available in 1936, that the minimum daily requirement is n less than 50 I. U. for infants and 200 I. U. for the average dult. Dani and Munsell ('37), in evaluating the adequacy of diets with respe to thiamin, have used 200 to 300 I. U. per adult per day as tentative standard. For average adults 400 to 500 I. U. per day regarded by Rose ('38) as a liberal intake. But Baker and Wrig ('36) claim that they have observed actual cases of beriberi in perso whose diets presumably contained over 300 I. U. per day. In a probability gastrointestinal absorption in these persons was very i efficient. Thus we see that present numerical standards are essential expressions of opinion inadequately substantiated by facts.

The question of numerical standards for children, pregnant ar lactating women, etc., is even more confusing than in the case

ormal adults. On the basis of present indications it would seem that fants, children, and adolescents require larger daily intake than lults in proportion to the caloric needs.

Sure ('28), Macy et al. ('30), and others have found that the itamin B" requirements of the rat for lactation are three to five mes the amount necessary for the support of normal growth in ung rats. Although milk is relatively low in thiamin, as compared ith whole grain cereals, Daniels and associates ('29) did not observe mptoms of thiamin deficiency in young rats suckled by mothers stricted to a milk diet. The young were debilitated but this was ributed to the inability of the maternal rat to ingest sufficient milk meet the caloric requirement of lactation. It is possible, however, at the maternal rats failed to ingest sufficient milk owing to poor petite caused by thiamin deficiency. The evidence undoubtedly ggests that women during pregnancy, and especially during lacta-n, should receive considerably greater amounts than normal adults. Since there is no reason to believe that this vitamin is injurious less ingested in amounts far beyond the range of probability, it ould be a safe practice, where conditions permit, for all classes of ople to take some rich source of thiamin in the diet.

The Extent of Thiamin Deficiency in Western Countries.—The neral experience of mankind shows that many different types of ts, forced upon people by reason of geographic and soil conditions, ve nourished them adequately in so far as the prevention of beriberi concerned. It is only in those regions where polished rice has been nsumed in excessive amounts, with consequent restrictions of the ake of other and better constituted foods, that this disease has n endemic. However, with the advent of the machine age, in-tive genius has perfected the technic of refining wheat, corn, and er cereals to a degree hitherto unknown. And in addition, in estern countries, the consumption of sugar has increased from a pounds per person per year a little over a century ago, to upwards 100 pounds per capita today. This increase is not uniform as pects individuals. Many adults eat sweets little or not at all, and ve no craving for sweet foods, but individuals who go to great remes in the consumption of sweet foods are numerous. It has n frequently pointed out during the past 25 years that, as a nation, are eating too large a proportion of our nutrients in the form refined and manufactured products. This practice constitutes a experience for the race, and there are numerous records which nonstrate that Western dietaries are frequently of poor quality in pect to several nutrients, among them, thiamin. As a basis for sideration of the problem, a few diets which have produced beri may be considered.

Chamberlain ('15) reported that among 5,000 Philippine Sc there were always 100 to 600 incapacitated from beriberi. Their consisted essentially of:

	Ounces Daily
Beef	12
White flour	8
Potatoes or onions	8
Polished rice	20

On changing the diet by substituting 16 ounces of unpolished and 1.6 ounces of beans for the 20 ounces of polished rice, and cluding 20 ounces of sweet potatoes, the number of cases of disease soon decreased to 50; during the following year to 3 ca and during the third year 2 cases, and thereafter none. Hehir (reported that during the siege of Kut-el-Amara, from December, 1 to April, 1916, the British troops ate white flour biscuits, canned m and fresh cooked horse flesh as their sole food. Many cases of beri appeared. No scurvy was diagnosed. The Indian troops used dha dry pulses of various kinds and either atta or barley flour, and refu meat. They were free from beriberi but suffered severely from scu In the light of our present knowledge the freshly cooked horse m protected the British against scurvy, and the unrefined cereals p tected the Indians against beriberi. Little ('12) called attention the frequent occurrence of beriberi in Newfoundland and attribu it to the excessive use of refined wheat flour. Aykroyd ('33) st that beriberi in Newfoundland is closely associated with pove The cheapest food there is white flour. The severity of winter all little opportunity for hunting and fishing, and most poor fami lay in a store of foods to last from November till June. If mone short, owing to a poor summer of fishing, they include more w flour and less meat, milk, peas, and potatoes. In May, after they h subsisted for six months on white bread and tea, and small ot additions, they develop beriberi. To cite a further example, Scott Herrmann ('28) described the occurrence of beriberi among farm in Louisiana. They produce animals for winter meat, chickens, eg milk, and vegetables of various kinds, but they sell these and b polished rice as their staple cereal. These farmers are rice grow and sell all their rice crop instead of keeping some of the unpolis grain for food. In autumn, it is said, they eat little else than polis rice and bacon grease, their "riz et sauce." When winter comes t add meat from home slaughtered animals, potatoes, and bread, a which no new cases of "maladie des jambes" (beriberi) occur. Th who have confidence in appetite as a guide to the selection of fo should note that these farmers eat their "riz et sauce" three time

y, virtually excluding all other foodstuffs except during the winter onths.

Cowgill ('34, l.c.) made a critical study of the "vitamin B" iiamin) content of typical human dietaries in different parts of e world, and concluded that deficiency of thiamin is relatively mmon. He recommended to physicians the consideration of thiamin erapy in gastrointestinal disorders, cardiac dysfunctions, various urological conditions, anemias, infant nutrition, anorexia, restriction growth, and in conditions of heightened metabolism. As shown ently by Vorhaus ('37) and others, thiamin has been used successly for each of these purposes.

Borderline States of Thiamin Deficiency.—McCollum has reatedly called attention to the tendency, in industrialized comunities especially, for wage-earning people to subsist largely upon ady to serve" or "grocery store" foods. Many foods of such selection semi-perishable products composed largely of refined cereals, gar, and slightly rancid fats. Moreover, they have been subjected high heat and are aged for a considerable time before they are d. Such foods tend to be deficient in all vitamins, and their liberal nsumption crowds out of the diet foods such as milk, fruits, vegeles, and fresh meats. The regular consumption of such staple foods ikely to bring about partial deficiency of any or all of the vitamins. health may result from a slight deficiency of thiamin, or any other ential nutrient, much less severe than is necessary to bring about typical syndrome associated with its complete absence from the t. Thus there are, for example, many degrees of iron or iodine rvation, recognizable as anemia or disturbance of thyroid function, pectively. It is with such borderline states of thiamin deficiency l their consequences that clinicians are now becoming increasingly cerned.

In human experience it seems certain that in most cases where amin deficiency occurs, but of a degree which falls short of ucing well-marked signs of beriberi, the diet is generally of a which causes multiple deficiency involving other vitamins, iron, ium, iodine, protein, or protein of poor quality, etc. In such cases remedy is not a source of thiamin alone but a better food supply. Minot ('36) has given us illuminating examples of patients whose rders arose from improper diet, but whose illnesses were extremely cult to diagnose correctly, because the description of their diets such as to suggest that they were satisfactorily nourished. But careful inquiry it was found that although a nutritious diet had n placed before them, they had been fussy and particular about ng it. Although they ate a little of many kinds of food, they had, long periods, selected principally dextrinized food, biscuits pre-

pared for invalids, and had eaten meat but scantily, while fruits
green vegetables had never been taken in more than minimal amou
the latter particularly in puréed form. The "tea and toast" die
notorious in the case of the chronically ill. Persons who suffer fr
digestive disturbances generally assert that a number of the be
class foods do not agree with them, and accordingly decline to
them. Thus a vicious cycle tends to become established. It has
become evident that nutritional deficiencies may arise even thou
the individual has an adequate diet. This is possible in conditi
of abnormal digestion, absorption, utilization, and metabolism. S
conditions are seen in chronic diarrhea, achylia gastrica, dysente
vomiting of pregnancy, or other causes.

Thiamin Craving in Rats.—Richter, Holt and Barelare ('
noted that rats deficient in thiamin ate dried baker's yeast with k
relish, and inferred that the incentive was due to its richness
thiamin, the ingestion of which relieved the deficiency. Followi
this they have demonstrated that such rats show an overmasteri
appetite for thiamin in the pure form. The odor as well as the ta
of thiamin aroused great interest, and the rats found at once t
bottle containing it in solution when as many as 12 containers w
available, furnishing different foods or solutions. Once they had tast
it they were avid in drinking the solution. One rat drank 11 cc.
5,500 I. U. in less than half an hour; another drank 29 cc. or 14,5
I. U. in 24 hours. Efforts to remove the bottle were met by fie
resistance, the bottle being held tightly with paws and teeth. Richt
and associates suggest that the craving for thiamin does not depe
upon the experience of a beneficial effect from taking it since t
ravenous appetite for it is immediately manifested when the nutrie
is placed before the deficient animals. Although this work is int
esting and important, it should not be concluded, on the basis of
that humans will select foods in accordance with their nutriti
requirements, even though free choice is provided. The higher i
telligence of humans is accompanied by all sorts of acquired ide
bizarre indeed in some instances, and powerful, which repr
"instinctive" demands if they potentially exist.

***"Vitamin B" Supplements in the Feeding of Infants a
Children.***—There are several direct experiments on record whi
support the opinion that typical dietaries of infants and childr
would be markedly improved by the use of some rich source
thiamin. It appears likely that anorexia which is prevalent in ea
years (Bartlett, '28), might be attributable in many instances to
inadequacy of thiamin, although faulty training and environment a
undoubtedly considerably responsible for "feeding problems."
stated several years ago by Schultz ('25): "A complaint for whi

child is brought to the physician more often than any other is sistent anorexia with cessation of gain of weight. . . . A problem o provide abundant vitamin supply, particularly the water soluble amin B." It will be recalled that Cowgill has shown that anorexia he first symptom of thiamin deficiency in animals.

Morgan and Barry ('30) reported a study of two groups including m 31 to 47 underweight children from 11 to 13 years of age who re compared as to growth in weight, height, and certain other sical indices of health over three periods, totaling 30 weeks. Each ld in one of these groups in each period was required to include the noon meal 3 ounces (85 gm.) of rolls made with 50 per cent eat germ and 50 per cent of white flour. The control group took usual white flour rolls; thus the former group ingested a total 5 ounces of wheat germ each week. The weight increases in each he wheat germ periods were about three times as great as in the trol periods. The difference in all cases was five or more times ater than the probable error of the difference. The height increases e significantly greater in two of the wheat germ periods than the corresponding control periods. These investigators point out t there may be a relation of the decreasing "vitamin B" content of modern diet to decreased appetite in children, nervousness, con- ation, and possibly other gastrointestinal disturbances. This view ears to be substantiated by the observations of Elias and Turner) that infants without extra "vitamin B" in the diet had a slightly ater incidence of anorexia than other infants fed supplements of eat germ and yeast.

Summerfeldt ('32) reported that when a group of normal children e given a special cereal mixture rich in "vitamin B" and the essen- mineral elements, in place of the commonly used cereals, during a week period, a marked increase in weight resulted. Ross and mmerfeldt ('35) substituted 3 ounces of a special cereal mixture in these nutrients for an equivalent amount of ordinary cereals he diet of normal children, and observed an increase in weight times the expected gain over a period of six months. However, did not attribute the beneficial effects exclusively to the high amin B" intake. In another experiment normal children fed a od" diet showed an increase in hemoglobin content during six ths from 10.4 to 10.7 gm. per 100 cc. of blood; whereas when tamin concentrate free from copper and iron was added to the the hemoglobin increased from 10.1 to 11 gm. Replacement of ordinarily used cereals by the special cereal mixture resulted in ncrease of hemoglobin from 10.2 to 12 mg. Their ordinary cereal sisted of oatmeal or cracked wheat. The special cereal consisted he same, but supplemented with 6 gm. of a "vitamin B" con-

centrate, made from wheat germ and brewer's yeast, for ea ounces.

It is obviously impossible to translate the present results of fe studies on infants and children into terms of thiamin since all o "vitamin B" sources that have been used contained other factors w might have affected the results. However, the available facts see suggest that thiamin is a significant limiting factor in the die many infants and children.

The Therapeutic Uses of Thiamin.—Conscientious and tho ful nutrition investigators regard the application of the knowled nutrition to clinical problems as a responsibility to be shared by the clinician and nutrition specialist. Without this attitude of mu dependence, mistakes of over-enthusiasm and misinterpretation of are likely to occur. It is hoped, therefore, that we can strictly m tain this essential point of view in the following brief discus which represents our interpretation as nutrition specialists of pre information and trends in certain clinical possibilities of thia Moreover, it is our intention that this view shall obtain in all o discussions in this book concerning clinical applications of mo nutrition.

The previous discussions have indicated the observable effect thiamin deficiency, and have reviewed the physiological action of nutrient, in addition to the normal bodily requirements and som the factors which affect the requirements for it. Thus the factual b are laid for the present consideration.

Vorhaus (l.c.) has recently reviewed the subject of thiamin ther and has differentiated the various clinical conditions in which vitamin appears to be of particular use from those in which its in large amounts is apparently without value. Some of the condit in which it has been employed with definite success are: neu associated with pregnancy; heavy metal neuritis, such as lead, arsenical neuritis occurring during intensive treatment for syph trigeminal neuritis; certain so-called diabetic conditions; cer alleged gastrointestinal disorders; and various types of anorexia, ticularly those associated with long standing illnesses. To this may be added the important observations of Weiss and Wilkins l.c.) and others, that thiamin gives striking improvement in cer types of cardiovascular dysfunctions. Also, the value of this vita in the treatment of alcoholic polyneuritis (Jolliffe et al., '36) the indications of its value in hyperthyroidism (Means, et al., must be mentioned. Space is too limited to discuss at length use of thiamin in these various conditions, but it is profitable to some attention to certain ones of them. The subject has been t oughly reviewed by Williams and Spies ('38).

Pregnancy.—Strauss and McDonald ('33) point out that it is tomary to refer any and all pathologic conditions developing ing pregnancy as manifestations of some occult toxemia, relief n which may be secured by termination of the gravid state. These olve the anemias, the polyneuritis, the vomiting, and eclampsia of gnancy. Whitfield (1889) appears to have first attributed poly- ritis of pregnancy to persistent vomiting. Berkwitz and Lufkin) reviewed the literature relating to 52 cases of polyneuritis of gnancy, and concluded: "The clinical and pathological picture of nerve changes in pregnancy is the same as that resulting from holism, infectious conditions, and diet deficiency disturbances such beriberi and pellagra. *The exact nature of the toxins producing ritis is still unknown.*" (Italics ours.) Strauss and McDonald clude from their studies that the polyneuritis of pregnancy is bably a dietary deficiency disorder similar to beriberi, and that rational therapy should aim at provision of the deficient nutrients, ecially the vitamin B complex. This view is supported by Plass Mengert ('33), Theobald ('36), Vorhaus ('37, l.c.) and Schwo- w ('37), all of whom have noted in many cases marked improve- nt or disappearance of the polyneuritic manifestations following min treatment.

The polyneuritis of pregnancy would seem to have its origin in eral causes or associated factors, among which are anorexia and niting of pregnancy. Plass and Mengert have pointed out that in forcing of high carbohydrate diets on patients with vomiting of gnancy there would seem to be increased risk of producing vitamin ciencies. Depletion of thiamin, of course, might be expected since ohydrate foods tend to increase the requirement for this nutrient.

Cardiovascular Dysfunctions.—Although Weiss and Wilkins , l.c., '37, l.c.) have reported striking effects from thiamin therapy everal cases of cardiovascular disturbance, it is not advisable to uss the subject here more than to emphasize the general cor- ondence between the heart symptoms and electrocardiographic in these clinical cases and those of experimental animals whose was deficient in thiamin. The question is naturally raised as to ther chronic deficiency of thiamin over a period of many years ht not be related, in part, to the high incidence of cardiovascular unctions occurring long before the end of normal life expectancy.

Alcoholic Neuritis.—Jolliffe and Colbert ('36) have reviewed the ature relating to the etiological factor in the development of neuritis in the alcohol addict. Thiamin deficiency was first sug- ed in this connection by Shattuck ('28). Later Strauss ('34) and kenhorn and Spies ('35) showed that patients with alcoholic itis improved if treated with a diet rich in several vitamins, even

while they were given one to two pints of whiskey daily. H Strauss concluded that alcohol, taken as whiskey, has no demonst toxic effect on the peripheral nerves. More convincing evidence thiamin deficiency is the etiological factor was furnished by Jo and Colbert, who obtained dramatic alleviation of polyneuritis in patient, and at least good response in another, treated with thiamin. Many other cases studied by Jolliffe et al., as reporte their numerous papers on the subject, have responded favorabl treatment with thiamin concentrates.

Cowgill's important work, which definitely establishes a d relationship between caloric intake and thiamin requirement, stitutes a basis for the explanation of the efficacy of thiamin in vention and cure of alcoholic neuritis. Since alcohol has a relati high caloric value and alcoholics tend to subsist haphazardly on f low in thiamin, the combination of these two antagonizing fac readily causes thiamin deficiency—consequently alcoholic neuritis.

Gastrointestinal Disorders.—Mackie and Pound ('35), am others, have discussed the changes in the gastrointestinal tract nutritional deficiency states. They emphasize that disease may re from a defective dietary or from abnormal physiology of the gas intestinal tract, and that defective absorption constitutes one of outstanding features of sprue, as was earlier pointed out by Ca and Rhoads ('32). Atrophy, resulting in impaired absorption, occ without extensive changes in the structure of the small intestine ap from the atrophy that is not disproportionate to the general wast of the body and the viscera. In a series of 75 cases of chronic ulcerat colitis they found evidence of nutritional deficiency in 63 per c Their appraisal of the diets indicated that the deficiency was multi including not only vitamins but also protein, and essential inorga elements. The changes which they were able to find in the intesti mucous membrane were those associated with edema, disorganizat of the normal motor activity, and reduction in tone of the intesti musculature.

Borsook and associates ('38) have reported their clinical experie with a series of 227 cases of chronic gastrointestinal malfunction wh were treated for periods of 3 months to 4 years by means of a spe dietary regimen. They eliminated starchy and sweet foods and structed the patients to take daily at least a pint of milk, 1 glass orange juice or tomato juice, 1 egg, 1 liberal helping of such vegetab as spinach, carrots, string beans, broccoli, lettuce or asparagus. T water in which the vegetables were cooked was added to the fo If more food was desired, it was allowed in the form of more mi meat, and vegetables. In addition, each patient was given a da supplement of 100 gm. of a cereal containing 10 to 15 per cent

t germ and the finely ground bran layer, and assaying 5 to 10 per gm. of thiamin. Also, it was rich in riboflavin and other mplex" factors. Since the improvement in gastrointestinal function including elimination, was so marked, they concluded that the s indicate the existence of a widespread partial "B complex" ency, and that most people require for normal gastrointestinal ion several times the minimum amounts of the water-soluble ins required to prevent severe deficiency diseases such as beriberi ellagra.

ut, as pointed out by Vorhaus (l.c.) symptoms of gastrointestinal nction are often vague and it is difficult to elicit a reliable y from patients with complaints of this sort. Hence, it is a em to secure objective and trustworthy evidence of the role of in in this class of disturbances. At best it would seem, from nt indications, that constipation, flatulence, etc., are not spelly attributable to thiamin deficiency, although it may accompany conditions.

kewise, the role of thiamin in treatment of anorexia is not clear. oubtedly many instances of poor appetite are attributable to an quacy of thiamin, but owing to the inability to clearly define or ure appetite, it is not possible to determine the role of this ent in the problem.

REFERENCES

ER (1913). "Beriberi." William Wood & Co., New York.
(1922). "The Vitamines." Williams & Wilkins Co., Baltimore.
BE (1881). Cited by Vedder.
(1903). Cited by Vedder.
ACH (1937). J. Am. Med. Assoc., *108*, 7.
IAN and ELVEHJEM (1936a). Biochem. J., *30*, 785.
ON and STONE (1937). Arch. Path., *23*, 207.
ERMAN and BURACK (1934). J. Exper. Med., *59*, 21.
EER and WENCKEBACH (1929). Wien. Arch. f. inn. Med., *16*, 193.
OMB (1930). Indian J. Med. Research, *17*, 721.
KEBACH (1934). "Das Beriberi-Herz. Morphologie, Klinik, Pathose." Julius Springer, Berlin.
and WILKINS (1936). Tr. Assoc. Med. Physicians, *51*, 341.
and MAPSON (1936). Nature, *138*, 27.
NS (1936). International Clinics, *4*, 120.
R and WILBUR (1937). Arch. Internal Med., *59*, 329, 512.
LL (1934). "The Vitamin B Requirement of Man." Yale U. Press, Haven.
L, DEUEL, PLIMMER and MESSER (1926). Am. J. Physiol., *77*, 389.
LL and GILMAN (1934). Arch. Internal Med., *53*, 58.

Sparks and Collins (1935). Am. J. Digest. Dis. & Nutr., *2*, 618.
Hargreaves, Fletcher and Dickson (1931). Trans. Roy. Soc. C
25, 197.
Eddy and Dalldorf (1937). "The Avitaminoses." Williams & W
Co., Baltimore.
Simpson (1936). Guy's Hospital Reports, *86*, 120.
Vorhaus, Williams and Waterman (1935). J. Am. Med. Assoc
1580.
Sherman (1934). "Food and Health." Macmillan Co., New York.
Cowgill (1921). Am. J. Physiol., *57*, 420.
Evans (1928). J. Nutr., *1*, 1.
Evans and Burr (1928). J. Biol. Chem., *76*, 263.
Evans and Bishop (1922). J. Metabolic Research, *1*, 355.
McCarrison (1919). Indian J. Med. Research, *6*, 275.
Weiss and Wilkins (1937). Ann. Internal Med., *11*, 104.
Hecht and Weese (1937). Klin. Wchnschr., *16*, 414.
Funk (1914). Ztschr. f. Physiol. Chem., *89*, 373.
Collazo (1923). Biochem. Ztschr., *136*, 278.
Rubino and Collazo (1923). Biochem. Ztschr., *140*, 258.
Kauffmann-Cosla, Vasilco and Oeriu (1932). Ztschr. f. Physiol. C
207, 113.
Lewinson (1937). Ztschr. Vitaminforsch., *6*, 209.
Roche (1931). Bull. Soc. chim. biol., *13*, 186.
Drummond and Marrian (1926). Biochem. J., *20*, 1229.
Lohmann and Schuster (1937). Naturwissenschaften, *25*, 26.
Peters (1936). Biochem. J., *30*, 2206.
Kinnersley and Peters (1929). Biochem. J., *23*, 1126.
Peters and Thompson (1934). Biochem. J., *28*, 916.
Johnson (1936). Biochem. J., *30*, 31.
Sherman and Elvehjem (1936b). Am. J. Physiol., *117*, 142.
Arakawa (1930). Tohoku J. Exper. Med., *16*, 118.
Chiba (1932). Tohoku J. Exper. Med., *19*, 486.
Uga (1935). Tohoku J. Exper. Med., *25*, 169.
Geiger and Rosenberg (1933). Klin. Wchnschr., *12*, 1258.
Platt and Lu (1936). Quart. J. Med., *5*, 355.
Wilkins, Taylor and Weiss (1937). Proc. Soc. Exper. Biol. & Med
584.
Tauber (1937). Proc. Soc. Exper. Biol. & Med., *37*, 541.
Lipmann (1937). Enzymologia, *4*, 65.
Tauber (1938). J. Biol. Chem., *123*, 499.
Lipschitz, Potter and Elvehjem (1938). Biochem. J., *32*, 474.
Westenbrink (1932). Arch. néerland. de physiol., *17*, 560.
Graham and Griffith (1932). Proc. Soc. Exper. Biol. & Med., *29*,
Leong (1937). Biochem. J., *31*, 367, 373.
Knott (1936). J. Nutr., *12*, 597.
Roscoe (1936). Biochem. J., *30*, 1053.
Harris and Leong (1936). Lancet, *i*, 886.
Carpenter and Sharpless (1937). J. Nutr., *13*, 235.

RE and BUCHANAN (1937). Proc. Soc. Exper. Biol. & Med., *33*, 77; . Nutr., *13*, 513.
RE and SMITH (1934). J. Nutr., *7*, 547.
ANS and LEPKOVSKY (1929). J. Biol. Chem., *83*, 269.
ANS and LEPKOVSKY (1931). J. Biol. Chem., *92*, 615.
ANS and LEPKOVSKY (1932). J. Biol. Chem., *96*, 179.
ANS and LEPKOVSKY (1935). J. Biol. Chem., *108*, 439.
LMON and GUERRANT (1930). Ala. Exper. Sta. 41st An. Report, 27.
HIPPLE and CHURCH (1936). J. Biol. Chem., *114*, cvii.
MMERER and STEENBOCK (1933). J. Biol. Chem., *103*, 353.
RE and BUCHANAN (1935). Proc. Soc. Exper. Biol. & Med., *33*, 75.
STENBRINK (1935). Arch. néerland. de physiol., *20*, 481.
MON and GOODMAN (1937). J. Nutr., *13*, 477.
HENRY (1937). J. Physiol., *89*, 287; Science, *86*, 200.
EBS and JOHNSON (1937). Biochem. J., *31*, 645.
NS, LEPKOVSKY and MURPHY (1934). J. Biol. Chem., *107*, 429.
IS and ZMACHINSKY (1937). Science, *86*, 245.
erican Medical Association, Council on Pharmacy and Chemistry 1936). J. Am. Med. Assoc., *106*, 1732.
NIEL and MUNSELL (1937). "Vitamin Content of Foods." U. S. Dept. gric. Misc. Pub., *275*.
E (1938). "The Foundations of Nutrition." Macmillan Co., New York.
ER and WRIGHT (1936). Proc. Roy. Soc. Med., *29*, 1145.
E (1928). J. Biol. Chem., *76*, 685.
CY, HUNSCHER, MCCOSH and NIMS (1930). J. Biol. Chem., *86*, 59.
FIELS, JORDAN and HUTTON (1929). J. Nutr., *2*, 19.
MBERLAIN (1915). J. Am. Med. Assoc., *64*, 1215.
HIR (1917). Mesopotamia Commission Report, Appendix III; also, roc. Asiatic Soc. of Bengal (1919), *15*, 212.
TLE (1912). J. Am. Med. Assoc., *58*, 2029.
ROYD (1933). "Vitamins and other Dietary Essentials." William Heinemann Ltd., London.
T and HERRMANN (1928). J. Am. Med. Assoc., *90*, 2083.
HAUS (1937). Am. J. Digest. Dis. & Nutr., *3*, 915.
OT (1936). New England J. Med., *215*, 1147.
ITER, HOLT and BARELARE (1937). Science, *86*, 354.
TLETT (1928). Am. J. Dis. Child., *35*, 26.
ULTZ (1925). Arch. Pediat., *42*, 347.
GAN and BARRY (1930). Am. J. Dis. Child., *39*, 935.
S and TURNER (1936). J. Pediat., *8*, 352.
MERFELDT (1932). Am. J. Dis. Child., *43*, 284;
and SUMMERFELDT (1935). Am. J. Dis. Child., *49*, 1185.
FFE, COLBERT and JOFFE (1936). Am. J. Med. Sci., *191*, 515.
NS, HERTZ and LERMAN (1937). Ann. Internal Med., *11*, 429.
LIAMS and SPIES (1938). "Vitamin B_1 and Its Use in Medicine." Macllan Co., New York.
USS and MCDONALD (1933). J. Am. Med. Assoc., *100*, 1320.
THFIELD (1889). Lancet, *i*, 627.

Berkwitz and Lufkin (1932). Surg. Gynec. & Obst., *54*, 743.
Plass and Mengert (1933). J. Am. Med. Assoc., *101*, 2020.
Theobald (1936). Lancet, *i*, 834.
Schwochow (1937). München. med. Wchnschr., *84*, 98.
Jolliffe and Colbert (1936). J. Am. Med. Assoc., *107*, 642.
Shattuck (1928). Am. J. Trop. Med., *8*, 539.
Strauss (1934). J. Am. Med. Assoc., *103*, 1.
Blankenhorn and Spies (1935). Trans. Assoc. Am. Physicians, *50*,
Mackie and Pound (1935). J. Am. Med. Assoc., *104*, 613.
Castle and Rhoads (1932). Lancet, *i*, 1198.
Borsook, Dougherty, Gould and Kremers (1938). Am. J. Digest. & Nutr., *5*, 246.

CHAPTER

XX

Riboflavin

Historical Development.—Until 1932 no one suspected that the natural water-soluble, yellow-greenish fluorescent pigments occurring in plants and animals were of unique significance in nutrition.

The first report of the occurrence of one of these was that of the yellow pigment of milk whey detected by Shoepf in 1784 (cited by Blyth, 1896) in a study of the composition and analysis of foodstuffs. In 1879 Blyth obtained this pigment from whey in the form of an impure resinous orange-red mass and named it "lactochrome." The first chemists to study the problem of isolation and identification of the yellow-green fluorescent pigment of milk whey were Bleyer and Kallman ('25). They obtained a crude preparation of this naturally occurring substance.

Isolation of the substance in pure form progressed slowly until the significance of yellow pigmented compounds obtained from whey (Bleyer and Kallman, l.c.) and heart muscle (Banga and Szent-Györgyi, '32) was indicated by the fundamental studies reported by Warburg and Christian ('32a), concerning a "yellow respiratory ferment" which they had prepared from bottom yeasts. These workers ('32b) succeeded in splitting the ferment into a protein component and a pigment component and noted that neither fraction alone was catalytically active.

In 1933 a number of naturally occurring, water-soluble, nitrogenous dyes had been isolated independently from various sources by different groups of investigators and their growth-promoting properties recognized. Ellinger and Koschara ('33) had separated from milk, liver, muscle, kidney, urine, yeast, and various plants three crystalline dyes, reddish-yellow in color, which were rich in nitrogen and oxygen and showed strong yellow-green fluorescence in aqueous solution.

They suggested that these compounds were related to Warbur Christian's oxidation ferment.

While attempting to isolate vitamin B_2 from whey and egg whi Kuhn and associates ('33) had separated in pure form nitrogenou water-soluble, yellow pigments displaying strong green fluorescen These dyes resembled spectroscopically Warburg-Christian's pigme obtained from yeast. They noted the similarity in distribution ar properties of vitamin B_2 and these yellow pigments and called atte tion to their probable relation to the yellow enzyme.

At the same time, Booher ('33, '34) also confirmed the fact th the "vitamin G growth promoting activity of whey is associated, least partially, with its water-soluble, yellow fluorescent pigment."

Nomenclature.—There is much confusion in the literature relatin to the vitamins of the B_2-complex. This state of affairs will probabl continue until the nature of all the components of this complex made clear.

Vitamin G and vitamin B_2 are terms which were employed t designate the heat-stable fraction of the B-complex after Smith an Hendrick ('26) had shown that the B-complex consisted of a heat labile and a heat-stable portion. Goldberger and coworkers ('26) named the latter the P-P (pellagra-preventive) factor. In America i has generally been called vitamin G, whereas in Europe the same substance was known as vitamin B_2. During the intervening years these terms have been employed to designate the antipellagra factor.

When Kuhn and his associates and other investigators proved by biological tests that the isolated yellow pigment is an indispensable nutrient, they applied the generic term "flavin" to these water-soluble yellow pigments isolated from various sources and grouped together under the term "lyochrome." Specific designation has been assigned to the flavin isolated from different compounds, e. g. lactoflavin from milk, ovoflavin from egg, hepatoflavin from liver, etc. Subsequent experiments definitely established that all these flavins are identical, and it appears that there is but one biologically important flavin. These investigators were wrong, however, in their assumption that flavin was B_2 or G, and in assigning the term B_2 to designate flavin they made the literature most confusing. There were two main reasons for this confusion—one was that B_2 and G in the minds of all research workers at that time meant the same as Goldberger's P-P or antipellagra factor, which the flavin is not; the second was that the rat which was the experimental animal employed in these studies presented a symptomatology of rat pellagra generally believed to be the analog of human pellagra. But this assumption was incorrect, for rat pellagra turned out to be caused by a deficiency of what is now known as vitamin B_6. Space does not permit the recording of

the details of this confusion in experimental data. The history of these erroneously interpreted experiments has been discussed by Sherman and Sherman ('37) and Hogan ('38).

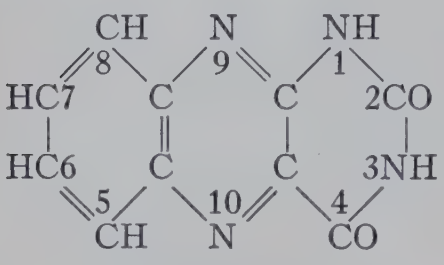
With the further investigation of the flavins and the elucidation of their chemical nature, The Council on Pharmacy and Chemistry of the American Medical Association ('37) proposed the use of the term d-riboflavin or simply riboflavin as the name for the nutritionally essential flavin and as a substitute for the terms vitamin G or vitamin B_2. This designation, based on the chemical nature of the substance, was later approved by the American Society of Biological Chemists and the American Institute of Nutrition. In Europe this substance is still generally called lactoflavin, irrespective of its source.

Isolation and Synthesis.—The isolation of riboflavin from many naturally occurring substances was first reported by Kuhn and his associates ('33, l.c.) and has been confirmed independently by the work of several laboratories. The methods of isolation used by different investigators are basically similar in principle. The general procedure consists of:—extraction with aqueous acid solutions, with alcohol, or with water-alcohol mixtures; precipitation or adsorption of the flavin (the adsorption methods are regarded as more reliable and greater yields are obtained); elution of the pigment with aqueous or slightly alcoholic mixtures containing alkaline compounds such as ammonia, pyridine, sodium hydroxide or diethylamine; removal of impurities from the eluate and concentration, precipitation, and purification by means of thallos or silver salts of the riboflavin.

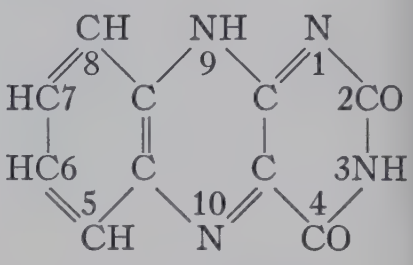
Warburg and Christian ('33) while studying their yellow enzyme or its pigment component isolated a photoderivative from the pigment of the yellow enzyme and had made clear many of its more outstanding properties. They observed that when the pigment component was exposed in alkaline solution to ultraviolet or ordinary light it underwent a decomposition in which the water-soluble substance was changed into a yellow photoderivative (lumiflavin) which is with difficulty soluble in water, but readily soluble in chloroform. They separated it by acidifying the solution at intervals and shaking with chloroform. This new pigment had the empirical formula $C_{13}H_{12}N_4O_2$. Its absorption spectrum resembled that of the original yellow one. The photoderivative readily lost its yellow color on reduction with hydrogen in the presence of palladium, and when warmed in a solution of barium hydroxide, it liberated urea.

Kuhn and Rudy ('34) followed up this observation and found that irradiation in alkaline solutions caused the splitting off of a hydroxyl-rich side chain, and the formation of Warburg and Christian's insoluble yellow lumiflavin. Since the original lactoflavin had the composition $C_{17}H_{20}N_4O_5$, and the photoderivative the composition

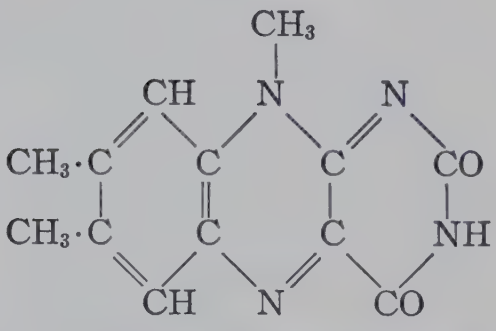
$C_{13}H_{12}N_4O_2$, it was evident that the hydroxyl-rich portion split by light must have the composition $C_4H_8O_4$. That there were in side chain thus split off four hydroxyl groups was shown by the f that lactoflavin formed a tetra-acetyl derivative. Booher ('38) reviewed the steps by which Kuhn and associates arrived at the c clusion that lactoflavin and lumiflavin belonged to the class of co pounds known as alloxazines, which had been discovered and stud by Kühling (1891). Another excellent review of the synthetic wo on the flavins is furnished by Turner ('34).



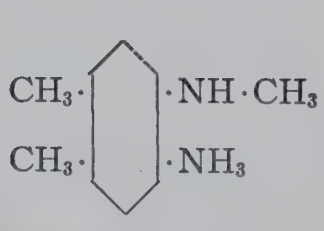
Alloxazine



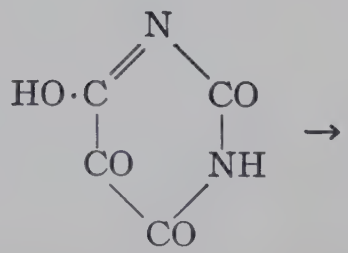
Isoalloxazine



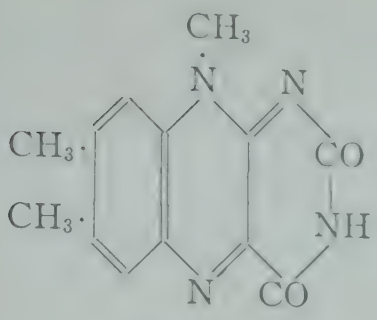
6, 7-dimethyl-9-methyl-isoalloxazine
Lumiflavin.



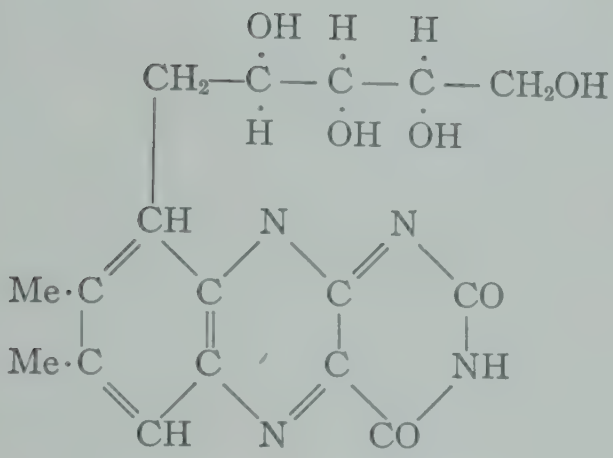
N-methyl-4, 5-diamino-o-xylene



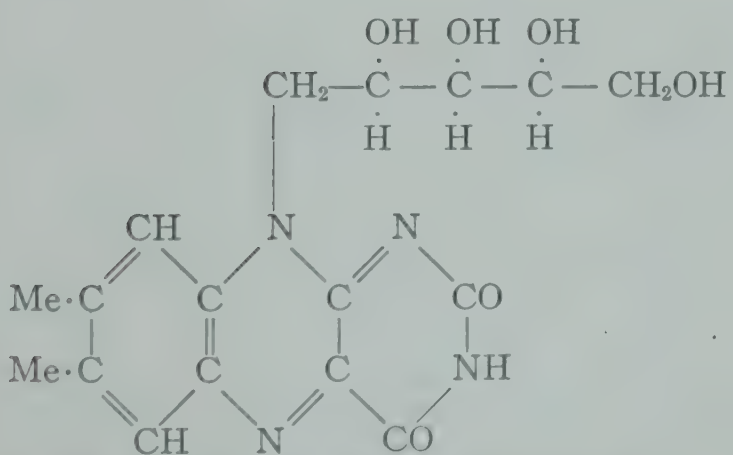
Alloxan



Lumiflavin



6, 7-Dimethyl-9-*l*-araboflavin
Araboflavin



6, 7-dimethyl-9-(*dl*'-ribityl)-isoalloxazine
Riboflavin

The synthesis of l-araboflavin and d-riboflavin was effected Kuhn and coworkers ('34, '35) by a method similar to that emplo in preparing lumiflavin, by starting with m-diamino-xylene, in w one hydrogen of an amino group was replaced by the sugar gr instead of a methyl group. It is of interest that the two appare inactive methyl groups in the nucleus of the flavin molecule essential for the biological activity of the compound. Both arabofl and riboflavin have been shown by animal assays to be biologic active, but the naturally occurring flavin from various foodstuf d-riboflavin. Araboflavin is only about one-third as potent as ribofla

A second synthesis of flavin compounds has been successf accomplished by Karrer et al. ('35a, '35b, '36) with results stantially in agreement with those of Kuhn. The methods emplo by Karrer for the preparation of the necessary intermediates somewhat different and give better yields. The final stage of synthesis is practically the same as that of Kuhn.

Chemical and Physical Properties of Riboflavin.—Ribofla crystallizes as yellow-brown needles which form clusters of slen prisms and is bitter to the taste. It has the empirical formula $C_{17}H_{20}N_4O_6$ and is a 6, 7-dimethyl-isoalloxazine with a sugar- (d-ribose) side chain in position 9, as represented above. The alloxazine nucleus is responsible for the color of flavin. The const tion of riboflavin demonstrates a relation to different groups of orga substances; namely, to the sugars, to the purines, to the azine p ments, and to benzene derivatives. It does not melt sharply darkens at 240° C. and decomposes at 267-294° C.

Riboflavin is soluble in water (25 p.p.m. at 25° C.) and sligh soluble in alcohol. It is insoluble in the usual fat solvents. When d-ribityl side chain has been split off by illumination in alkal solution, the resulting methyl derivative, lumiflavin, is soluble in solvents. Riboflavin is relatively highly heat-stable. It is sensitive change in alkaline solution at elevated temperatures but is stable even strong acids. It is not altered by atmospheric oxygen or ot oxidizing agents including the halogens. Riboflavin is "reversi reduced," that is, it takes up hydrogen from reducing agents a gives it up easily under the influence of atmospheric oxygen. It destroyed by visible and ultraviolet light. In neutral or acid solutio irradiation produces lumichrome, which is like lumiflavin except t the 9-methyl group is replaced by hydrogen. Lumichrome exhi an intense blue fluorescence. Aqueous solutions of riboflavin are yell in color and show yellow-green fluorescence in natural light. T fluorescence is at a maximum between pH 6 and pH 7 and is marke intensified when examined in the ultraviolet rays.

Riboflavin, lumiflavin, and Warburg's yellow enzyme show w

efined absorption maxima which are closely similar. The maxima for boflavin are at 220, 267, 366, and 446 millimicrons. The green fluoescence lies in the region from approximately 500 to 630 millimicrons. Riboflavin yields with sodium bisulfite, sodium hydrosulfide, zinc ust and acid, or hydrogen and platinum, a leuco-derivative which verts to the original dye on oxidation.

The Nature and Function of Riboflavin.—The relationship tween certain vitamins of the B-complex and some enzymes is of ime importance in metabolism. Several of the enzymes which are ncerned in the oxidation-reduction reactions of the cells are congated proteins in which the conjugating or prosthetic groups con- in one of the vitamins of the B-complex. In each of these cases the tamin, or a molecule of which the vitamin is a part, has been upled with a protein carrier. The combination exhibits either zymatic activity or, in certain instances, may be a member of a stem which is necessary for the activation of an important enzyme. iboflavin is a member of the flavin system, an important oxidative ain in the organism.

The Warburg-Christian yellow oxidation pigment was shown by e researches of Kuhn et al. ('36), Theorell ('34a, '35a), and others consist of two parts: (a) a flavin phosphoric acid capable of dergoing reversible oxidation and reduction, and (b) an enzyme- e protein component with which the flavin phosphoric acid is in imate association. Warburg now gives the name "flavin enzyme" the combination of these two components.

Warburg ('34) has demonstrated that riboflavin is the active group the yellow oxidation enzyme which appears to be present in vary- g amounts in practically all living cells and tissues. Its vitamin ivity is believed to be due to the formation of riboflavin-phosphoric er (Rudy, '35; Theorell, 34b, 35b; Kuhn and Rudy, '35; Theorell al. '35). This ester, also known as the yellow coenzyme, forms with specific protein (Theorell, '35a, l.c.) the yellow oxidation enzyme hich has a significance in cell respiration. Flavin is present in certain sues such as liver, kidney, heart, etc., in the form of the phosphoric er bound to protein, while in substances such as milk it is believed exist in the free form.

Recently, Corran and Green ('38) isolated a flavin-protein comund from cow's milk. The colored prosthetic group of the comund can be split off from the colorless protein. A solution of the sthetic group exhibits the characteristic yellow-green fluorescence flavin. The absorption spectrum is practically identical with that of oflavin. The prosthetic group does not seem to be riboflavin, but dence indicates that it may be the corresponding phosphorylated mpound. From their observations Corran and Green believe that

milk flavoprotein acts catalytically not by serving as an intermed between reduced coenzyme and the carrier, but by forming a com with the reductant and the oxidant and thus facilitating the tran of hydrogen.

Riboflavin is an important factor actuating cell combustion p esses as the oxidation of sugars. It also complements the i containing respiration enzyme. Its growth-promoting property partially or totally destroyed by the photochemical transformation flavin by visible light into the blue fluorescent photochrome.

Evidence indicates that riboflavin is not synthesized by the ani cell; consequently it must be supplied by the diet. Hence the colla of animals depleted of riboflavin is, no doubt, due to the cellu asphyxiation resulting from the lack of a cellular oxidation catal

Development and Symptomatology of Riboflavin Deficiency the Rat.—The production of an uncomplicated riboflavin deficie was very difficult owing to the confusion existing as to the preparat of a diet free from this factor but adequate in all other respects. T symptomatology produced by the lack of riboflavin in the diet still not entirely clear.

A survey of the early literature shows that Goldberger and Li ('26), who were attempting to produce pellagra in the rat, w probably the first to describe the symptoms of riboflavin deficie in the rat. The main symptoms encountered in their experime were arrest of growth; following this, a tendency of the lids of o or both eyes to adhere together with, in some cases, an accumulati of dried secretion on the margin of the lids. Subsequent to or at t time of the appearance of the ophthalmia, alopecia developed in m of the rats, leading in some of the animals to almost complete denu tion of the head, neck, and trunk. The denuded areas were shar outlined and bilaterally symmetrical. In addition to these as describ in Chapter XXI the rats on this experimental diet also showed characteristic dermatitis, stomatitis, and other pellagra-like symptor It is now apparent that most of the symptoms of the rat disease wh the early investigators attributed to a lack of the P-P factor w due to a deficiency of riboflavin. Further work (Hogan and Hunt '28; Hogan and Richardson, '33, '34; György, '34; Chick et al., ' Harris, '35) definitely established that the vitamin G (B_2) comp was composed of several factors, of which riboflavin was one.

The Bourquin-Sherman diet ('31) has been used for studies riboflavin deficiency. Some investigators believe that this method bio-assay measures the riboflavin activity because the basal rati contains ample B_1 and therefore the growth response that follo the addition of the material under assay is an indication of the rib flavin content. However, this is not a justifiable deduction since oth

factors in the B-complex affect growth in rats and this method is a measure of the growth-promoting activity in rats of the vitamin G (B_2) complex content of substances. The diet used by Richardson and Hogan ('36) appears to be well adapted for this purpose.

György first ('35) used basal diets devoid of the water-soluble vitamins except the chemically pure vitamin B_1 (thiamin). When this diet was supplemented by riboflavin the rats still showed the characteristic dermatitis which was promptly cured by other supplements. The work, therefore, demonstrated that riboflavin has no dermatitis-preventing activity and hence plays no role in rat pellagra. Bender et al. ('36) further noted that flavin does not prevent or cure dermatitis in the rat.

P. L. Day and associates ('31) described the development of cataract in rats, mice, chickens, and monkeys deprived of vitamin G (B_2). The condition begins with the appearance of a conjunctivitis and keratitis after 7-8 weeks on the diet, followed by a dullness of the eyeball and finally a definite opacity. The incidence of cataract in their experiments was almost 100 per cent. This group of investigators ('37) have presented evidence pointing to riboflavin as the substance that prevents this type of cataract.

Bourne and Pyke ('35) repeated Day's work and produced cataract in but 31 per cent of his animals. On the other hand, neither György ('35, l.c.) nor Richardson and Hogan ('36, l.c.) observed cataract in their rats deprived of riboflavin. The reason for the divergent results is not apparent. It seems probable that Day's observations are correct and that the diets of the experimenters who failed to produce cataract may not have been completely depleted of riboflavin.

György's ('38) most recent observation is that riboflavin deficiency in the rat is often associated with pediculosis and that administration of riboflavin to the lice-infested animals is followed by complete disappearance of the parasites. This phenomenon does not seem to be a result of a generally diminished vitality of the rats preventing their keeping themselves clean, for most of these lice-infected rats were neither weakened nor inactive. This may be an indication that flavin is essential in the normal sensory acuity of the skin, and if in flavin deficiency skin sensitivity is reduced, the rats are not able to keep themselves free from pediculosis in the manner of normal animals.

Riboflavin Deficiency in the Dog.—Sebrell ('29, '33) appears to have been the first to recognize as a distinct pathological entity a condition in dogs which has since been shown to be due to deficiency of riboflavin. Lillie and Sebrell ('37) described the pathology of the disease under the name "yellow liver." It occurred in certain but not all of their dogs fed diets designed for the study of the etiology of

blacktongue. Sebrell and Onstott ('38) have shown conclusively th "yellow liver" is due to riboflavin deficiency.

The symptoms characteristic of the acute attack of this deficien state developed rapidly, and death occurred within a few hours. The was a sudden onset of weakness and ataxia, and very soon the anim was unable to stand or move its legs. There were at this time va ing degrees of spasticity, which was sometimes marked. The do appeared to be fully conscious, since they followed activity in the roo with their eyes and attempted to wag their tails and move their hea There was no evidence of pain or discomfort. Bradycardia and exaggerated sinus type of cardiac arrhythmia (the heart speeding on inspiration and slowing on expiration) probably due to vagoton occurred. The respiration was slow and regular. Within an hour th animal had usually passed into a deep coma, in which there was no r sponse to stimulation. It remained in this condition for several hou until death supervened. These workers did not note survival beyon 12 hours unless the animal was given riboflavin. Preceding deat the heart slowed and the respirations became shallow and labore Death appeared to be caused by respiratory failure.

Pathologically the most striking changes in riboflavin deficienc in this animal occurred in the liver. This organ was regularly yello in color, with fine red lobular markings. It was firm, usually quit friable and often distinctly greasy to the feel. There was no evidenc of enlargement. The yellow color and fine red markings were ev dent on the cut section. The liver cells were filled with fat droplet In the kidneys, fatty infiltration of the epithelium of the limbs o Henle's loops, which they state are often seen in normal dogs, wa practically constant in "yellow liver" animals. Other pathologica findings in their animals were less constant and may have been du to complications arising from incipient blacktongue (dog pellagra or from former attacks of this disease. In the brain, injection of th meninges was seen in about half the animals. Severe, diffuse, quit general fatty degeneration of the white substance and the cor disintegration of the chromophil corpuscles (tigrolysis) in the brain stem ganglia, nerve-cell atrophy and perinuclear edema in th basal ganglia and cortex were observed in a sufficient number of dog to indicate that these changes are probably a feature of the deficienc state due to lack of riboflavin.

Riboflavin in the Nutrition of the Chick.—Hauge and Carric ('26) were the first to definitely demonstrate that in addition to th antineuritic substance (thiamin), the chick required a growth promot ing factor for normal growth and development. Norris and associate ('29-30, '30-31, '33, '36), and Bethke, Record and Kennard ('30-31 '33) confirmed and extended the work of Hauge and Carrick. Thes

studies showed that the requirements of the growing chick for the growth promoting factor now known to be riboflavin are very great.

From their experiments Norris and associates ('36, l.c.) concluded that chicks need approximately 290 micrograms of riboflavin per 100 gm. of feed in order to attain normal weight at the age of 8 weeks. Hens require about 230 micrograms per 100 gm. of feed in order to produce eggs that will hatch well. As small an amount as 130 micrograms is sufficient for normal egg production. They found that the rate of growth in chicks is closely correlated with the amount of riboflavin ingested, which indicates that this pigment is intimately associated with growth processes. This is easily visualized on the assumption that each increment of new tissue must have in it a certain quota of Warburg's yellow respiratory pigment. These investigations also show that, for the chick, ordinary components of rations for growth employed in America and Europe do not provide enough riboflavin for development, hence riboflavin-rich supplements must be provided. Lack of such information has, in the past, caused enormous economic loss to poultry producers.

Riboflavin and Pellagra.—There are considerable data indicating that riboflavin is not concerned in the etiology of pellagra. Treatment of pellagrins with riboflavin was unsuccessful (Dann, '36; Fouts et al., '36). However, riboflavin deficiency may occur in man simultaneously with or independently of pellagra.

Storage and Excretion.—Kuhn, Kaltschmidt and Wagner-Jauregg ('35) found that the riboflavin content of animal organs cannot be increased to any degree even by feeding ten times the normal amount. These investigators made the interesting observation that in rats deficient in B_6, but receiving an abundance of riboflavin, the liver contained, on a weight basis, about 30 times as much riboflavin as muscle. Another noteworthy observation is that the riboflavin content of the liver is affected by the length of the period of flavin deprivation. The longer the rats were kept on the B_6 deficient diet, supplemented with this nutrient, the lower the liver riboflavin values became. There was no change in the flavin content of the control animals.

The organism apparently holds tenaciously to its store of riboflavin, for even in rats that die as a result of flavin deprivation, the content of this substance in the liver, kidney, and heart still remains about one-third of the normal level (Kuhn et al., '35, l.c.; Vivanco, '35). When flavin is added to the diet of depleted animals, growth starts before the reserves in the various organs are restored to normal.

Under adequate dietary conditions riboflavin is a normal excretory product. The total excretion of flavin in the urine and feces of the rat ranges from 3 to 5 micrograms daily, the feces containing about

twice as much flavin as the urine (Vivanco, l.c.). After a 14-da period of total deprivation of "vitamin G (B_2)," no flavin was e creted in the urine and growth was suspended.

Emmerie ('36, '37a) has conducted an extensive investigation riboflavin excreted by man. He found the daily output of this sub stance in the urines of normal men to vary between 819 and 12 micrograms. However if the intake of the nutrient was greatly in creased, there was a corresponding increase in the amount excrete When the consumption of flavin was considerably decreased, th excretion exceeded the intake; with an increased intake, there wa both an increased retention and excretion. Roscoe ('36) reported simi lar findings. The rate of destruction of riboflavin by the mammalian organism is not known; experimental data indicate, however, tha some destruction occurs.

Biological Activity of Riboflavin.—The data from several labora tories showing the growth increment in the rat with various intakes of riboflavin are so little in accord that they do not afford a basis for establishing a quantitative relationship.

Kuhn and associates ('33, l.c.) using the Bourquin-Sherman "vita min G (B_2)" deficient diet ('31, l.c.), supplemented by vitamin B_4, found that 7 micrograms of riboflavin supported growth at the rate of 3 gm. per rat per week. Ansbacher and associates ('36) found that when rats were fed a basal diet free from all water-soluble vitamins, supplemented with thiamin and a B_6 concentrate and varying amounts of riboflavin, the rate of growth differed with the amount of riboflavin fed. With daily doses of 5, 10, and 20 micrograms, weight increases during a period of 49 days were 0.26, 0.57, and 0.77 gm. respectively. With an intake of 3 micrograms crystalline riboflavin per rat daily, Booher ('38, l.c.) secured weight increases of 3.1 gm. per week. Von Euler and coworkers ('34) using a different diet found that daily ad ministration of 5 and 10 micrograms of riboflavin induced an average daily gain of 1.1 gm. and 1.3 gm. respectively. Itter, Orent and Mc Collum ('35) fed 100 micrograms daily and obtained weight increases of 1.1 to 1.4 gm. per rat daily. Lepkovsky and associates ('35) using 100 and 300 microgram supplements of flavin daily observed increases of 2.9 and 3.0 gm. per day respectively. Stare ('35) found a daily dose of 50 micrograms of the nutrient resulted in a daily gain of about 0.6 gm. Karrer et al. ('33) feeding 3 micrograms of the substance reported a daily increase of 0.75 gm. per animal. Kuhn and associates ('35, l.c.) state that 10 micrograms daily of natural riboflavin induced a daily gain of 1.45 gm. and a similar dose of the synthetic product re sulted in a gain of 1.43 gm.

Riboflavin Assay.—Chemical and physical methods for the de termination of riboflavin have been developed (Morton, '35; Supplee

et al., '36; Weisberg and Levin, '37, Emmerie, '37b). The fact that iboflavin exhibits a blue-green fluorescence in ultraviolet light serves as a basis for its determination by fluorometric methods.

The various methods of estimating riboflavin consist of the following processes:—extraction of the flavin with water, dilute alcohol, and dilute acetone; adsorption on fuller's earth and elution with dilute pyridine; estimation of the riboflavin content of the eluate by comparing the fluorescence with that of solutions of known riboflavin content or by converting the riboflavin to lumiflavin by exposing it in alkaline solution to the action of light and then determining the lumiflavin colorimetrically. The existing methods need still further refinement, for the chance of loss of material at the various steps of these procedures is considerable. Therefore the results thus obtained are undoubtedly low and must be considered only approximate.

Measurement of riboflavin by biological means has been conducted by a number of investigators, but in only one case (Whitnah et al., 37) was the flavin content of the foods compared with that of pure iboflavin.

There is no officially recognized procedure for the assay of riboflavin. The important factor in the biological assay of this nutrient s the selection of a basal diet absolutely devoid of flavin and the nclusion in this ration of all the other essential factors of the vitamin B-complex free from riboflavin. Some of these factors are now available for this purpose in highly purified forms. The rat at he weaning age is the animal commonly used. The feeding of such basal diet will cause cessation of growth, one of the earliest symptoms of the deficiency, and if the deprivation is continued the characteristic denudation will appear. When the flavin-containing material is added the animals begin to grow and the denuded areas show ew fur.

A biological method of assay using young chicks has been described by Norris and associates ('36, l.c.). Norris as a result of these xperiments defines a chick unit of riboflavin as one microgram. However there is as yet no recognized unit of riboflavin.

Dietary Requirement for Riboflavin.—The riboflavin requirements of the animal organism appear to be dependent on the size nd activity of the body tissues. Reliable data for the absolute amounts f riboflavin required for normal nutrition are lacking at this time. Stiebeling ('36-'37) and Rose ('37) using the Bourquin-Sherman nit of vitamin G which is regarded by them as the riboflavin unit, estiated the normal human requirement of riboflavin. Stiebeling proosed 450 units for boys under 6 and girls under 7 years of age, 540 nits for boys from 7 to 10 and girls from 8 to 13 years of age, and 600 nits for older children and adults. Rose has suggested for children

up to 10 years of age at least 400 units a day or 20 units per 1 calories if more than 2000 calories a day are consumed; for adults s recommended 20 units per hundred calories. However, the Bourqui Sherman unit as a measure of riboflavin is, at least, equivocal as w have discussed previously in this chapter.

Distribution.—Riboflavin is most abundant in milk, egg whit liver, and leafy vegetables; other good sources are heart, pancrea beef muscle, veal, chicken, apricot and tomato. Lesser amoun are present in grains and legumes. Yeast, although probably the ric est natural source of the vitamin B-complex, varies in its flavin co tent according to the particular sample. Fish liver contains but litt of this substance and fish muscle is almost devoid of it.

The retina of fish eyes contain flavin and von Euler and Adl ('34) suggested that this flavin may be of special significance i vision.

REFERENCES

Shoepf (1784). Dissertation. Cited by Blyth "Foods, their Compositio and Analysis." 4th ed. C. Griffin Co. (1896).

Blyth (1879). J. Chem. Soc., *35,* 530.

Bleyer and Kallman (1925). Biochem. Ztschr., *155,* 54.

Banga and Szent-Györgyi (1932). Biochem. Ztschr., *246,* 230.

Warburg and Christian (1932a). Naturwissensch., *20,* 688, 980. b. Bio chem. Ztschr., *254,* 438.

Ellinger and Koschara (1933). Ber. deutsch. chem. Gesellsch., *66,* 315 808, 1411.

Kuhn, György and Wagner-Jauregg (1933). Ber. deutsch. chem. Gesell sch., *66,* 317, 576, 1034, 1577.

Booher (1933). J. Biol. Chem., *102,* 39.

Booher (1934). J. Biol. Chem., *107,* 591.

Smith and Hendrick (1926). U. S. Pub. Health Reports, *41,* 201.

Goldberger, Wheeler, Lillie and Rogers (1926). U. S. Public Healt Reports, *41,* 297.

Sherman and Sherman (1937). Ann. Rev. Biochem., *5,* 342.

Hogan (1938). J. Am. Med. Assoc., *110,* 1188.

Council on Pharmacy and Chemistry (1937). J. Am. Med. Assoc., *108* 1340.

Warburg and Christian (1933). Biochem. Ztschr., *266,* 377.

Kuhn and Rudy (1934). Ber. deutsch. chem. Gesellsch., *67,* 892, 1298.

Booher (1938). J. Am. Med. Assoc., *110,* 1105.

Kühling (1891). Ber. deutsch. chem. Gesellsch., *24,* 2363.

Turner (1934). Ann. Rep. on the Progress of Chem., The Chem. Soc. London.

Kuhn and Weygand (1934). Ber. deutsch. chem. Gesellsch., *67,* 2084.

Kuhn, Reinemund and Weygand (1935). Ber. deutsch. chem. Gesellsch. *68,* 1765.

KARRER, SCHÖPP, BENZ and PFAEHLER (1935a). Helvet. chim. acta, *18*, 69.
KARRER, BECKER, BENZ, FREI, SALOMON and SCHÖPP (1935b). Helvet. chim. acta, *18*, 1435.
KARRER and MEERWEIN (1936). Helvet. chim. acta, *19*, 264.
KUHN, RUDY and WEYGAND (1936). Ber. deutsch. chem. Gesellsch., *69*, 2034.
THEORELL (1934a). Biochem. Ztsch., *275*, 37.
THEORELL (1935a). Biochem. Ztschr., *278*, 263.
WARBURG (1934). Naturwissensch., *22*, 441.
RUDY (1935). Naturwissensch., *23*, 286.
THEORELL (1934b). Biochem. Z., *275*, 466.
THEORELL (1935b). Biochem. Z., *279*, 186.
KUHN and RUDY (1935). Ber. deutsch. chem. Gesellsch., *68*, 383.
THEORELL, KARRER, SCHÖPP and FREI (1935). Helvet. chim. acta, *18*, 1022.
CORRAN and GREEN (1938). Nature, *142*, 149.
GOLDBERGER and LILLIE (1926). U. S. Pub. Health Reports, *41*, 1025.
HOGAN and HUNTER (1928). J. Biol. Chem., *78*, 433.
HOGAN and RICHARDSON (1933). J. Biol. Chem., *100*, lv.
HOGAN and RICHARDSON (1934). J. Nutr., *8*, 385.
GYÖRGY (1934). Nature, *133*, 498.
CHICK, COPPING and EDGAR (1935). Biochem. J., *29*, 776.
HARRIS (1935). Biochem. J., *29*, 776.
BOURQUIN and SHERMAN (1931). J. Am. Chem. Soc., *53*, 3501.
RICHARDSON and HOGAN (1936). Res. Bull., 241, Missouri Agric. Exper. Sta.
GYÖRGY (1935). Biochem. J., *29*, 741.
BENDER, ANSBACHER, FLANIGAN and SUPPLEE (1936). J. Nutr., *11*, 391.
DAY, LANGSTON and O'BRIEN (1931). Am. J. Ophth., *14*, 1005.
DAY, DARBY and LANGSTON (1937). J. Nutr., *13*, 389.
BOURNE and PYKE (1935). Biochem. J., *29*, 1865.
GYÖRGY (1938). Proc. Exper. Biol. & Med., *38*, 383.
SEBRELL (1929). U. S. Pub. Health Reports, *44*, 2697.
SEBRELL (1933). Nat. Inst. of Health Bull., No. *162*, Part 3, p. 23.
LILLIE and SEBRELL (1937). Nat. Inst. of Health Bull., No. *162*, Part 4, p. 37.
SEBRELL and ONSTOTT (1938). U. S. Pub. Health Reports, *53*, 83.
HAUGE and CARRICK (1926). J. Biol. Chem., *69*, 403.
MORRIS, HEUSER, WILGUS and RINGROSE (1929-1930). Poultry Sci., *9*, 133, 378.
MORRIS, HEUSER, WILGUS and RINGROSE (1930-1931). Poultry Sci., *10*, 93.
MORRIS, HEUSER, WILGUS and RINGROSE (1933). Fifth World's Poultry Cong., Rome, Proc., *2*, 512.
MORRIS, HEUSER, WILGUS and RINGROSE (1936). Bull. 660, Corn. Univ. Agric. Exper. Sta.
SETHKE, RECORD and KENNARD (1930-1931). Poultry Sci., *10*, 355.
SETHKE, RECORD and KENNARD (1933). Poultry Sci., *12*, 332.
MANN (1936). J. Nutr., *11*, 451.

Fouts, Lepkovsky and Helmer (1936). Proc. Soc. Exper. Biol. & M
35, 245.
Kuhn, Kaltschmidt and Wagner-Jauregg (1935). Ztschr. f. phys
Chem. *232,* 36.
Vivanco (1935). Naturwissensch., *23,* 306.
Emmerie (1936). Nature, *138,* 164.
Emmerie (1937a). Acta brev. Neerland., 7, Nos. 4 and 5.
Roscoe (1936). Biochem. J., *30,* 1053.
Ansbacher, Supplee and Bender (1936). J. Nutr., *11,* 401.
v. Euler, Karrer, Adler and Malmberg (1934). Helvet. chim. acta,
1157.
Itter, Orent and McCollum (1935). J. Biol. Chem., *108,* 579.
Lepkovsky, Popper and Evans (1935). J. Biol. Chem., *109,* liv.
Stare (1935). J. Biol. Chem., *111,* 567.
Karrer, Schöpp, Benz and Pfaehler (1933). Helvet. chim. acta, *18,*
Morton (1935). "The Application of Absorption Spectra to the Study
Vitamins and Hormones." London.
Supplee, Ansbacher, Flanigan and Hanford (1936). J. Dairy Sci.,
215.
Weisberg and Levin (1937). Ind. Eng. Chem., Anal. Ed., 9, 523.
Emmerie (1937b). Proc. Intern. Congr. Tech. Chem. Ind. Agr., *1,* 57.
Whitnah, Kunerth and Kramer (1937). J. Am. Chem. Soc., *59,* 11
Stiebeling (1936-1937). (Cited by Sherman and Lanford (1938). J. A
Med. Assoc., *110,* 1278.)
Rose (1937). "Laboratory Handbook for Dietetics." Ed. 4, Macmill
Co., New York.
v. Euler and Adler (1934). Ztschr. f. physiol. Chem., *228,* 1.

CHAPTER

XXI

Pellagra and Animal Deficiency Diseases Related to Pellagra

IS NO EASY MATTER to trace far back the history of indubitable lagra, because in the past it was confused with many other diseases h as scurvy, leprosy, syphillis, erysipelas, etc.

Don Gaspar Casal, the learned and able physician of King Philip Fifth of Spain, as early as 1735 was the first to grasp the individuality and significance of pellagra. In Italy it was first recognized Pujati in 1740, but nothing was published until 1771 when Frapolli scribed it under the popular name of "pellagra," by which it has g been known to the peasantry. In France the disease was first erved by Jean Hameau in 1818 and in Rumania by Theodori 1833. The history of the discovery of pellagra in other countries ws everywhere again and again the same late and fortuitous recogion, notwithstanding its antiquity and wide distribution of the ease. Until about 1907 pellagra was looked upon as non-existent in rth America and was not even mentioned in medical text books. t it existed, and two physicians in different sections of the country, ey in Utica, New York, and Tyler in Summerville, Massachusetts, d both recorded in 1864 one case each. But it was not until 1907 t pellagra which began to develop with alarming speed in the uthern States became a subject of inquiry in the United States. merous and severe cases were observed in the Austrian Tyrol ween 1875 and 1905. The first description of pellagra in England eared in 1912. Sporadic cases occur in many countries and epinics have been observed in France, Spain, Italy, the Balkan counrs, and in Egypt. At present the largest number of cases of pellagra ur in the southern parts of the United States, where 200,000 are d to be afflicted. The mortality is also higher in the United States n abroad.

Pellagra occurs among all races. Although not inherited the ease may affect children at an early age; the larger number of tients, however, develop the symptoms between the close of the sec and fifth decade of life. It occurs in both sexes, but in the Un States the subjects of the disease are in larger proportion mar women. This suggests a relationship between pregnancy and lactat as predisposing influences.

Pellagra is a disease of poor people in the rural districts who s sist on a limited choice of food. It is rarely seen among the well-to who enjoy a more varied diet. It is seasonal in its outbreaks, 90 cent of the cases having their onset in the period from April to Ju with remissions in the late fall and winter. The duration of the att is anywhere from 6 weeks in acute cases to 20-30 years or even lon in the chronic forms. It is generally confined to warm countries. B the southern portions of Europe and the United States have suffe the most.

The most typical symptom is erythema, which at first resemb sunburn but later becomes brown and makes the skin rough a scaly. It attacks only certain parts of the body surface, particula the backs of the hands in adults and of the feet in children. Oth parts not infrequently attacked are the sides or front of the nec the face, elbows, or knees. Another marked peculiarity is its tenden to appear at about the same time on both sides of the body. Accor panying the skin eruption are soreness of the mouth, redness of t tongue, indigestion and diarrhea, as well as disturbances of t nervous system leading in the severest cases to insanity. On the who the pathology of this disease is not distinctive and does not offer mu aid in determining the possible cause of the disease.

The etiology of pellagra is still not entirely clear. A number views have been proposed to explain its cause. Marzari more than century ago was the first to express the belief that it resulted fro a dietary insufficiency. He observed that the pellagrins in Italy us excessive amounts of maize and he believed that maize lacked suf cient albuminous material to be a good food.

The two theories which have had in the past the greatest numb of adherents were the maize toxin theory and the infection theor According to some proponents of the toxin theory, the toxin was pr duced by molds and other microorganisms with which maize ma be infected. According to others, a toxic substance was produced the animal body as a result of digestion and absorption of mai products. However, the experimental work supporting these theori is confusing and difficult to interpret. In more recent years Joblin and Arnold ('23) in the United States observed an increase in t fungus, hyphomycetes, in pellagrins and supposed that these mol

ere in themselves toxic or produced a toxin when grown on corn. hey isolated from laboratory cultures of fecal fungi from pellagrins fluorescent dye which sensitized albino rats to light so that exposure used erythema, thickening and scaling of ears and other exposed in areas. They were never able to identify these molds as a causa- ve factor in pellagra. No further work appears to have been done nce, hence it is doubtful as to how much significance may be at- ched to these findings. Some of the objections to the toxin theory e as follows: a toxin is not apt to cause recurring seasonal attacks ng after the patient has ceased to take it in his food; the attacks pellagra continue to recur in pellagrins after they have ceased to t corn. The disease also occurs in persons who do not eat corn who have eaten it but rarely.

The infection theory has little to substantiate it although extensive idemiological studies have been made. Exhaustive studies of pel- grous material have yielded no evidence that an infectious agent as associated with the disease, and thorough efforts to transmit the sease from man to man have also failed.

In 1910 Sambon, an Italian, after a number of investigations both Italy and France, advanced the parasitic theory stating that the dis- se is due to an infection with some living organism probably trans- itted and propagated by an infected sand fly. During the period of 13-1914, the Thompson-McFadden Commission made an epidemio- gical study of pellagra in Spartansburg County, S. C. Their investi- tions included an examination of the dietaries of pellagrins d non-pellagrins of this region. Their conclusions from this survey ere that there is no relation between the character of the diet and cidence of the disease. As a result of these observations they sug- sted that the stable fly displayed certain characteristics which seemed qualify it as a vector of pellagra.

The objections to the infection theory are the failure to find in e blood or in the tissues and body fluids of pellagrins any parasites specific bacteria and the failure to reproduce the disease when the ood of pellagrins is injected into the body of monkeys or healthy men.

The modern era of pellagra research began in 1913 when Gold- rger of the U. S. Public Health Service undertook an investigation the disease in the South. The epidemic was extremely widespread d the morbidity high (in 1917 170,000 cases were recorded). Gold- rger and associates ('15) first planned three sets of investigations nich he conducted at the Carolina State Asylum, the Georgia State sylum, and the State Sanatorium at Jackson, Mississippi. During ese surveys he noted that the staffs of these institutions although ing in the same buildings and supposedly getting the same dietaries ere immune from pellagra. It seemed to be only the inmates who

were afflicted with the disease and died of it. His first concl was that since the nurses, doctors, orderlies, and other membe the staff in spite of the fact that they handled the patients and lived and slept in the same wards with the pellagrins, appeared immune, the disease could not be contagious and was not infect In addition, he had observed that the difference between victim the disease and those who were not afflicted with it was that pella consumed diets mainly composed of cereals and almost devoi animal proteins. He therefore looked into the matter of the diet of the asylum staffs and inmates and found that theoretically groups were supposed to get the same food, but actually, the staff getting the choice foods of the dietaries and furthermore, the members also obtained additional foodstuffs from outside sou which privilege was not enjoyed by the patients. Thus it was parent that the diets of the two groups were different.

Next Goldberger and his coworkers ('15, l.c.) performed a clusive experiment in an orphanage in Mississippi where pellagra rife,—recurring with regularity every spring. In this institution, children from 6-12 years of age were receiving the poorest type foodstuffs. Those below that age were given in addition a glass milk daily; whereas, those older than 12 had a more varied diet si they earned a little money to supplement their diet with more ch foods. Goldberger added liberal quantities of eggs and milk to orphanage diet and the next spring there was no reappearance pellagra in the institution, whereas in other similar ones where dietary change was made, the recurrence rate of the disease was high as in previous times. Goldberger and his staff followed experiment with a series of similar ones to discover what fo actually prevented pellagra. They established the fact that lean m milk, eggs, yeast, tomato juice, tinned salmon, and a number other foods if given in sufficient quantity will prevent the disea Then ('15a) he attempted to induce pellagra by dietary means healthy men. On promise of a pardon, 11 convicts on the Ran Prison Farm volunteered to serve as subjects. These men were stricted to a diet composed of maize meal, white wheat flour, potat salt pork, and syrup. At the end of 6 months, 7 of them were fou suffering from the disease. The addition to this diet of 30 gm. yeast, 200 gm. of meat, or 2 pints of milk daily cured the pella and prevented its recurrence.

Subsequently, Goldberger set out to determine the manner which these foods exercise their protective function. What factor they supply which is lacking in maize? Goldberger ('22), and a Wilson ('21) and Bigland ('20), who worked in Egypt, believing protein of corn to be of poor biological value, first thought that

gra was caused by an amino acid deficiency. Zein, one of the pro-
ns of maize, is deficient in tryptophane and lysine and the pellagra-
eventive foods are rich in these amino acids. However, the discovery
at a yeast extract practically devoid of amino nitrogen was as
ective in curing pellagra as was yeast, meat, and milk, put an end to
at theory.

To finally settle the question of infection, Goldberger ('16) and
s associates then tried a number of ways to transmit pellagra to
emselves by inoculations with blood, nasopharyngeal secretions,
es, urine, and desquamating epithelium. But the results were en-
ely negative. This, therefore, served as further proof that pellagra is
t an infectious disease.

Spencer ('16) was the first to suggest that spontaneous canine
acktongue was analogous to human pellagra. The first report of
perimental pellagra appeared in 1917, when Chittenden and Under-
l produced by dietary means a condition in dogs strikingly sugges-
e of pellagra in man. Chittenden ('07) had observed and described
is syndrome as early as 1905 in connection with his nutritional
dies on protein, but he did not, at that time, recognize its relation
pellagra. This pellagra-like syndrome was characterized by a sud-
n loss of appetite and marked apathy. After refusal to eat for a day
two the dogs developed a severe stomatitis, characterized by pustules
the inner surfaces of the cheeks, lips, and edges of the tongue,
hich simulated a mass of rotten flesh. The odor was foul, and
e mucous lining of the mouth could be wiped away by stroking it
th absorbent cotton. There was intense salivation and bloody diar-
ea. Pustules on the thorax and upper abdomen were characteristic.
autopsy of these animals, which died early from convulsions, the
ief feature was an intense hemorrhagic condition of the large in-
tine and ulcers of the duodenum. This pathologic condition was
oduced by feeding a diet of cooked peas, cracker meal, and cotton-
d oil. A similar condition was produced, but with greater diffi-
lty, by a diet of meat, cracker meal, and lard. In continuing the
dy, Underhill and Mendel ('25, '28) found that some substance
esent in butter fat, egg yolk, or carrots cured this condition. Cod
er oil was not effective, neither was yeast nor meat, but crystalline
otene cured the disease. Cary ('20) was the first to suggest that
e Chittenden-Underhill syndrome was identical with the canine
ease known as blacktongue.

Wheeler, Goldberger and Blackstock ('22) described the pathology
a disease in dogs which had been variously designated as black-
ngue, typhoid in dogs, and canine diphtheria, which closely re-
nbled the syndrome of Chittenden and Underhill. They had dis-
vered this disease at various places in the South and they expressed

the belief that it represented the analog of human pellagra. T quoted several observers who had met with the condition in dog widely separated areas.

In 1926 Goldberger and Lillie found that the typical diet of p grins would cause blacktongue in dogs. They also produced ex mental blacktongue in dogs by means of a diet adequate in respects but deficient in pellagra-preventive foods. This impor finding opened the way to the effective study of the etiology of lagra in man. They cured this experimental condition by feed yeast and red meat. Experimental blacktongue is similar to the bl tongue that occurs spontaneously in dogs, which in turn is sim to human pellagra as to its geographical distribution, symptomatol and histology. The stomatitis produced by Underhill and Mer ('25, l.c.) corresponded to these but a number of the symptoms the Underhill-Mendel dogs were different; scrotal lesions and dermatitis of the chest characteristic of Goldberger's dogs and spontaneous blacktongue were absent from the Underhill-Men animals. Since carotene completely controlled and prevented Underhill-Mendel syndrome and since proof of the identity of two conditions such as histologic studies is lacking, it seems proba that the two types of diseases are essentially different though gros similar in certain respects. The Underhill-Mendel syndrome see to be very similar to the combined deficiency of vitamin A a B-complex which has been described in man by Wright ('32).

Smith ('32), in a review of the literature referring to oral spi chetes and related organisms in fusospirochetal disease, points out th this group of organisms, which grow so profusely in the mouths sick dogs, is capable of producing severe infection in normal anim when inoculation is made with minimal trauma. He showed th there is true symbiosis of these organisms as the causative factor Vincent's angina, pyorrhea, and fusospirochetal disease of the lun Miller and Rhoads ('35) observed the characteristic flora described blacktongue in dogs. They were unable to infect normal dogs inoculating masses of these organisms under the labial mucous me brane. Smith and associates ('37) produced both the Underhill-Me del syndrome and Goldberger's blacktongue in dogs and conclud that the clinical and bacteriological findings in dogs fed these di are identical. They demonstrated that fusospirochetal organisms ex in enormous numbers in the oral lesions of dogs on both diets. Th attach significance to this observation because similar organisms found in the lesions of human pellagra, and because upon treatme of both blacktongue and pellagra these organisms disappear. In ad tion, this seems to be further proof of the analogy between expe mental blacktongue and human pellagra.

It appears that the primary deficiency of the Chittenden-Underhill-Mendel dogs was vitamin A and that of Goldberger's dogs was the antipellagra factor. The mouth condition which was regarded by both groups of investigators of dog pellagra as clinically significant, was in fact due to overgrowth of the mucous membranes by the fusospirochetal flora as the result of debility of the animals. This observation is of additional outstanding importance since it makes clear the fact that microbial agencies, which in the well individual are not able to establish themselves upon mucous surfaces and become a menace to health, may easily do so when the vitality is lowered through malnutrition. In this case the overgrowth of fusospirochetal organisms occurred equally menacingly in two distinct deficiency states.

In his dietary studies, Goldberger observed that most foods rich in vitamin B would cure pellagra. Consequently when in 1926, Smith and Hendrick showed that vitamin B was made up of at least two substances, one thermolabile, the other thermostable, Goldberger et al. ('26) repeated these experiments on rats and confirmed Smith's observations. Then they proceeded to test the effect of the thermostable portion of the vitamin B-complex in the form of autoclaved yeast and water extracts of autoclaved yeast on both blacktongue dogs and cases of human pellagra and obtained beneficial results. Goldberger therefore designated this fraction of the vitamin B-complex as the P-P (pellagra-preventive) factor.

The Role of Sunlight in Pellagrous Dermatitis.—The relationship of pellagra to light has frequently been commented on in the clinical literature. In the early days, sunlight was believed by some to cause the disease. However, the sunshine was neither hotter nor different in the pellagrous regions than in other sections of the world where the disease did not exist; and furthermore even in the early days, it has been observed that the dermatitis may occur on parts of the body covered by clothing. In those exposed to direct sunlight the dermatitis in pellagra seems to appear earlier in relation to the other symptoms. Various experiments have been performed with fenestrated gloves which seemed to show protection from the dermatitis when the parts were protected from light. The clinical experience of Smith and Ruffin ('37) has shown that the dermatitis of the exposed surfaces of human pellagrins is closely dependent upon the action of sunlight on the patient's skin. Spies ('35) recently has also studied this phase of the disease and has reported that pellagrous lesions occur in the absence of sunlight or ultraviolet radiation. Spies emphasized that pellagra should be considered a systemic disease and not a cutaneous condition and that under certain circumstances, all matters of conjecture, sunlight might act as an irritant and precipitate the cutaneous lesions of the disease. The role of light in

pellagra is extremely confusing but it is certain that the influenc the solar light is only of minor importance,—a patient must first h the disease internally before the sun can cause or influence the matitis. The deficiency state and not the sun is the cause of the lagrous dermatitis.

Rat "Pellagra" and Vitamin B_6.—Goldberger and associ ('26, l.c.) found that the typical pellagra-producing diet of man l wise caused in rats the development of a syndrome characterized arrest of growth, ophthalmia, some denudation of hair, and derma at one or more of the following sites: ears, front of neck and up part of chest, forearms, backs of forepaws, shins, and the backs the hind paws. The matted fur on the backs of the hind paws desc mated, leaving a denuded, pale pink, glistening skin. In some instan there was fissuring or ulceration at the angles of the mouth, toget with a lesion at the tip of the tongue, beginning as a small roug circular, grayish opacity or bleb; or as an ulceration, which in so animals went on to the formation of a localized yellowish slou Inflammation of the anterior part of the floor of the mouth a diarrhea were also seen in some animals.

Goldberger et al. ('26, l.c.) found that lean meat as well as ye or yeast extract would cure the condition in rats. They were a able to clear up this syndrome, like blacktongue in dogs, by t provision of an earth adsorbate of yeast extract which had be freed from the antineuritic substance (thiamin) by heating. Go berger concluded that in both the dog and the rat the lesions observ represented pellagra and that the deficiency was prevented by t same substance which had a preventive or curative effect in hum pellagra. It appeared then that these deficiency states in the th species must have a common origin.

In 1927 Boas described in rats a syndrome caused by feeding C nese egg white as a dietary source of protein, the chief characterist of which were eczematous dermatitis, accompanied by alopec blepharitis, spasticity, and in some cases edema of the feet and hem rhages in the skin. The similarity of this condition to pellagra w noted. The efforts to discover the nature of this egg white injury h been discussed in Chapter VI.

Even as recently as 1934 the great difficulty in making furth progress in the study of the etiology of pellagra lay in the fact th there were several water-soluble vitamins which had not been clea distinguished as to their effects, and had not been prepared in su cient purity to permit the producing of separate and distinct d ficiency states due to each of these factors. The first advance this direction came through the discovery by Kuhn and associates (s Chapter XX) that riboflavin is an essential nutrient, and a co

onent of the B-complex. As has been mentioned in the discussion of iboflavin, Kuhn found that when rats which were restricted to the Sourquin-Sherman type of diet had ceased to grow, and were approaching the rat-pellagra stage, the provision of crystalline riboflavin nduced a prompt growth response. This they wrongly interpreted to nean that riboflavin possessed intense vitamin B_2 (G) effect. György '34) found that rats, under these dietary restrictions, develop pellagra-ke symptoms even when liberally provided with riboflavin. György '37) describes the symptoms of this uncomplicated deficiency as nvolving symmetrical dermatitis of the peripheral parts of the body, ne paws, nose, mouth, and ears. There is no pruritus. Scaliness and dema are evident. Alopecia usually does not occur; at most it is slight. bscesses form, particularly around the mouth and cheeks. Sub-ngual necrotic ulcers are not uncommon. In advanced stages scaliness ver the trunk is seen, and slight or no loss of hair accompanies the ale formation. The symptoms appear in rats in 6 to 15 weeks. It now ecame possible to prepare diets containing two known factors in ne water-soluble, or B-complex group, and so to make further prog-ss in characterizing the remaining ones.

Birch, György and Harris ('35) made a critical study of the re-onse of rats and dogs to experimental diets designed to show hether these species react alike or differently when different foods preparations were the source of vitamin B_2, this term now being ed to signify those essential nutrients of the old B-complex other an thiamin and riboflavin, both of which had become available in ure form for experimental purposes and were supplied in all diets. hey knew, on the basis of clinical literature, that pellagra is essen-ally confined to maize-eating communities; that molasses is one of e principal constituents of the human pellagra-producing diet; that h has preventive value in pellagra and in blacktongue; and that dministration of riboflavin does not benefit human pellagrins. On e basis of these facts they prepared experimental diets which early led to the following conclusions:

Maize, which forms a large part of the diet of pellagrins, is highly fective in preventing or curing the condition hitherto called rat llagra, rat dermatitis, or rat acrodynia. Pellagra and blacktongue-oducing diets contained considerable of the nutrient required to event rat dermatitis, hence the rat syndrome is etiologically distinct om human pellagra. In conformity with the earlier suggestion of örgy ('34, l.c.), the antidermatitic substance needed by the rat was led vitamin B_6.

György ('35) showed that thiamin together with riboflavin failed prevent dermatitis in rats. Peters' eluate (Kinnersley et al., '33) on other hand contained the factor that prevents rat pellagra.

Thus by 1935 it was established that the vitamin G (B_2) com was composed of riboflavin, a growth-promoting substance, and I dermatitis-preventive factor essential for the rat but different f the P-P factor required by man. Vitamin B_6 has no effect on bl tongue in dogs or on the so-called chick-pellagra. Dann and Subar ('38) reported further confirmation of the characteristic compon of the vitamin G (B_2) complex. György et al. ('34, l.c.) and Edgar Macrae ('37) believe that vitamin B_6 may be identical with facto of Chick and Copping ('30). (See Chapter XXII.)

Dann ('36) confirmed the B_6-deficiency theory of rat derma and further showed that rats on a B_6-deficient ration develop der titis as readily in the dark as in the light, which is further evide that this syndrome is not analogous to the dermatitis of human lagra.

György ('38a) has reported that environmental factors, such temperature, may seriously affect the appearance of symptoms vitamin B_6 deficiency. During the summer when the temperat ranged from 80°-100° F. the incidence of rat acrodynia was decide on the decline. With lower temperature conditions the producti of the acrodynia was increased. Acceleration in the producti of the dermatitis was achieved by exposing rats to low tempe ture (40° F.). In these experiments a high food intake was char teristic of the rats exposed to cold without a corresponding increase weight. In spite of its earlier appearance, the acrodynia in the r exposed to cold progressed slower and exhibited certain peculiarit such as prevalence of erosions of the lips and angles of the mou and the extremities exhibited the signs of deficiency at a compa tively later stage of the disease. Peters' eluate cured and prevented t disease.

György calls attention to the possible clinical significance of the results. Striking similarity between this disease in the rat and chilbla in man suggests that vitamin B_6 reserves in the body may functi in the predisposition to chilblain, and that vitamin B_6 treatme might be used in chilblain. He also calls attention to the similar of the angular stomatitis to that of perlèche common in British Ind Aykroyd and Kushman ('36) concluded that the etiology of t human disease lies in a nutritional deficiency, probably a factor the B_2-complex. György suggests that, in the light of his observatio the etiology of perlèche should be correlated to B_6 deficiency.

Birch ('38) presented evidence pointing out that two facto are concerned in rat pellagra. One is the water-soluble vitam B_6 and the other is fat-soluble and is found in the fatty acid fracti of maize oil. This factor appears to be similar to Burr and Burr's "fat acid factor" and to the fat-soluble antidermatitis factor of Hogan a

ichardson. He suggests that the physiological function of vitamin B_6 connected with the utilization of the unsaturated fatty acids.

Vitamin B_6 and Microcytic Hypochromic Anemia in Puppies.—outs, Helmer, Lepkovsky and Jukes ('38) produced severe microcytic ypochromic anemia in puppies by feeding them a synthetic diet con-ining thiamin, riboflavin, the chick filtrate factor, and nicotinic acid, nd which was apparently deficient only in vitamin B_6. Addition of $_6$ to the experimental diet cured the anemia.

The Isolation of Vitamin B_6.—A number of experimenters have tempted the isolation of vitamin B_6. The chemical studies of Birch nd György ('36) indicate vitamin B_6 to be of a basic nature (although contains no primary amino group) and probably attached as a rosthetic group to protein, since it is not easily extracted from tissue ntil after digestion.

Keresztesy and Stevens ('38a) report the isolation of B_6 from rice ran. It is a nitrogenous base, the hydrochloride of which is freely luble in water and sparingly in alcohol and acetone. They describe e compound as white platelets melting at 204-206° C. with decom-osition. Daily doses of 0.050 mg. caused disappearance of symptoms B_6 deficiency. Daily doses of 0.100 mg. were highly effective both relief from symptoms and in stimulating growth.

György ('38b) showed that vitamin B_6 can be adsorbed upon ller's earth and eluted by barium hydroxide. It is precipitated by osphotungstic acid. He reports the isolation of crystalline material hich, in doses of 15 micrograms per day cured rat acrodynia in 2 eeks. These crystals are described as colorless rods of varying size, ith rounded ends, which have a tendency to form rosettes or fan-aped groups.

Lepkovsky ('38) announced the isolation in crystalline form of ctor 1, which is the portion of the B-complex other than thiamin d riboflavin that is absorbed by fuller's earth under specified condi-ns. The crystals are described as colorless rods which aggregate ostly as rosettes and sometimes fan shapes. The dry crystalline mate-l has a slight yellowish tinge which is believed to be due to some purity. No analysis of the crystals is given. Dermatitis of the periph-l parts of the body of rats, involving the feet, paws, ears, and as around the mouth, were promptly cured by daily doses of 10 crograms of crystalline factor 1. When growth had ceased on the perimental diet causing these symptoms, there was an immediate owth response when the crystalline material was administered. om the symptomatology described, it appears that Lepkovsky was ling with what others call B_6 deficiency, and that it is this factor ich he has isolated.

In a later paper Keresztesy and Stevens ('38b) further describe the

chemistry of this vitamin. The empirical formula is $C_6H_{12}N_3O$ (does not contain O-CH_3 or N-CH_3 groups. The free base mel 160° C. and has the empirical composition of $C_6H_{11}N_3O$. Both base and its hydrochloride readily sublime. With ferric chloride vitamin gives a reddish-brown coloration. The pure substanc stable to concentrated hydrochloric acid at elevated temperature is not affected by heating with alkalies, by nitrous acid, ethyl nit or Fehling's solution.

Absorption spectrum studies of the substance in acid, alkaline, neutral solutions show that it has tautomeric properties. Well def absorption occurs in the spectral region from 2300 to 3300 Å. In hy chloric acid at pH 2, there is a single band with maximum absorp at 2925 Å. At pH 4.5 this band diminishes in intensity along with a band with maximum absorption at 3275 Å. At pH 6.75 the latter b has increased markedly, while that at 2925 Å. has disappeared, simultaneously a new band at 2560 Å. has appeared. At pH 10.2 two bands which were present at pH 6.75 have increased in inten and shifted toward the shorter wave lengths.

Thus we may confidently expect that full knowledge of the st ture of B_6 will be soon forthcoming, and that its synthesis will not long delayed.

Chick Pellagra and the Filtrate Factor.—In 1931 Ringrose, No and Heuser described a pellagra-like syndrome in chicks which w restricted to diets containing commercial casein, purified casein, dried raw egg whites together with cereals, cod liver oil, and necessary mineral supplements. Granulation of the eyelids, a visc exudate which caused the lids to stick together, crusty scabs at mouth angles, which later extended around the nostrils and un the mandible, were characteristic of this disease. The skin on the sc and between the toes thickened and cornified. Cracks and fissures a developed.

In 1932 Kline and coworkers reported that chicks fed a diet co posed of yellow corn, casein, wheat middlings, cod liver oil, NaCl a $CaCO_3$ which had been heated dry at 95-100° C. for 144 hours, p duced chick pellagra in about 3 weeks.

Ringrose and Norris ('36) noted that dried pork liver contain a factor which prevents the development of the pellagra-like conditi in chicks in addition to another factor required for growth. The f mer they found to be insoluble in an alcohol-water solution and be destroyed by dry heating in air at 100° C. for 144 hours or at 12 for 50 hours, and by autoclaving at pH 11 but not at pH 9. Th growth promoting factor was soluble in alcohol-water solution a was stable to heat in a dry atmosphere and relatively stable to au claving at pH 11. Yeast, dried or autoclaved, and liver extract w

neffective in preventing the syndrome caused by the egg white or asein diet. Dried pork liver and the residue of extracted liver, on ne other hand, were completely effective in this respect.

In 1936 Lepkovsky and Jukes succeeded in separating the chick ermatitis factor from the rat dermatitis factor. The term filtrate factor ney provisionally used to indicate the water-soluble vitamin which revents the dermatitis in chicks produced by feeding a dry heated ation of natural foodstuffs as described by Kline et al ('32, l.c.).

The richest known sources of the chick filtrate factor are yeast and ce bran. It is also found in egg yolk, beef, and in small amounts in ereal. This substance may be extracted with water-saturated butyl cohol and 95 per cent ethanol. Jukes ('37) found that it is also easily xtracted by hot or cold acidified water. This factor is stable to auto-aving and labile to dry heat at 120° C. for 24 hours. It is resistant to xidizing and reducing agents. It is stable to benzoylation and is ardly precipitable by phosphotungstic acid. The rat antidermatitis ctor (B_6) is readily adsorbed on fuller's earth, whereas the chick trate factor remains behind. This filtrate factor prevents or cures ick dermatitis.

At this time the evidence is insufficient as to the relationship be-veen the chick filtrate factor and the pellagra-preventive factor. Fouts al. ('36) reported successful treatment of pellagra in man with a rice an concentrate containing the chick filtrate factor. Koehn and vehjem ('36) cured blacktongue with a liver extract which was also fective for chick pellagra. Sebrell and associates ('37) observed that a eparation containing the filtrate factor also contained the black-ngue preventive factor. However the distribution of the chick filtrate ctor and the P-P factor shows marked differences. Jukes' (l.c.) says indicate that this nutrient is present in the same quantity in ef, whole corn meal, and wheat germ. Corn meal on the other hand an extremely poor source of the P-P factor, whereas beef and wheat rm are good pellagra-preventive foods. This suggests that the chick-llagra factor is distinct from the pellagra-preventive factor of man. rther experimental evidence is necessary before final conclusions n be made.

The Human Pellagra-Preventive or Blacktongue Factor.—The idence that vitamin G (B_2) was made up of several factors was of l in determining the relation of the various pellagra-like animal de-iency diseases to human pellagra. It has been demonstrated, as ed in the discussions above, that the rat pellagra-preventive factor d the chick pellagra-preventive factor are not analogues of the dis-se in man.

Recent experimental data lead to the assumption that the pellagra-eventive factor is the blacktongue preventive also. Ruffin, Persons,

Harvey and Smith ('37) have observed that extracts of liver used parenteral administration contain a factor which alleviates bl tongue symptoms, but which is only partially effective in curing disease. Sydenstricker and Thomas ('37) observed that the addi of the liver residue, which is usually discarded in the prepara of the extracts, produced a curative effect in blacktongue. Pellag reacted similarly to the same fractions (Ruffin et al., '37, l.c.).

These observations offered additional evidence supporting the ic tity of the blacktongue-preventive factor with the pellagra-preven factor.

During their study of chick pellagra Elvehjem and associates (isolated nicotinic acid from potent liver concentrates. They correla all the known facts concerning the properties and distribution of n tinic acid and tested the effect of nicotinic acid and nicotinamide dogs suffering from blacktongue.

Immediately upon the announcement by Elvehjem and associ ('37, l.c.) that nicotinic acid could cure blacktongue in dogs, sev investigators proceeded to test its effectiveness in human pella Smith and associates ('37) described a dramatic cure of a case pellagra by the daily administration of 60 mg. of nicotinic acid. Th was striking improvement of the appetite within 24 hours. T mental confusion began to improve after 48 hours, and the pati was entirely rational after 6 days' treatment. Within 3 days there v noticeable change in the skin of the face, and after 12 days the s was normal. On the seventh day there was a striking change in electrocardiogram, the T waves having returned to normal. On twelfth day the abdominal and cremasteric reflexes were present.

Fouts and collaborators ('37), Harris ('37), and others have a confirmed the value of nicotinic acid in the treatment of pella Spies et al. ('38) reported the cure of 11 cases of pellagra by administration of nicotinic acid. Three of these were alcoholic pella 2 were endemic pellagra, and 6, pellagra secondary to organic dise The symptoms which they noted as clearing up promptly were g sitis, stomatitis, ptyalism, vaginitis, urethritis, proctitis, the char teristic dermatitis, and the peripheral neuritis. The recent studies Spies, Bean and Stone ('38) and Bogart ('38) are further support these observations. Chick, Macrae, Martin and Martin ('38) h cured a pellagra-like condition in swine by nicotinic acid.

The conclusion seems secure that nicotinic acid or nicotinic a amide is either a part or the whole of Goldberger's P-P factor.

Woolley and associates ('38) studied effectiveness of various p dine derivatives in the treatment of dogs suffering from blacktong Their experiments demonstrate that a specific structure is requi for antiblacktongue potency. Nicotinic acid N-methyl amide, ni

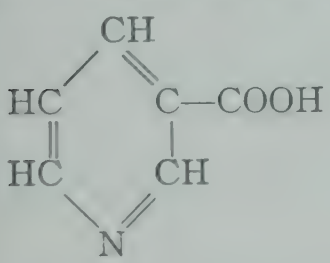
ic acid N-diethyl amide, nicotinamide glucosidoiodide, and nico-
uric acid were effective. Wooley et al. suggest that in addition to
otinic acid and its amide the compounds possessing antiblack-
gue activity are those which are capable of oxidative or hydrolytic
version to these substances in the animal organism.

Although nicotinic acid, or its amide, is ineffective in rat pellagra
d chick pellagra, it is essential for the health and growth of rats
ehjem and Frost, 37; György, '38c; Euler and Malmberg, '36)
d pigeons (Funk and Funk, '37).

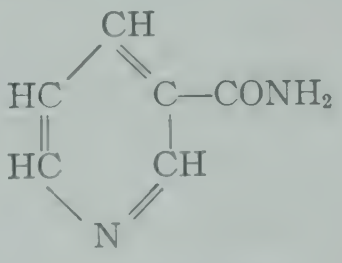
Further data pointing to nicotinic acid in the form of its amide
an important constituent of the living cell are the experiments
nonstrating that this factor is also indispensable for the growth
various microorganisms (Knight, '37).

Nicotinic Acid and Nicotinamide as the Antipellagra Vitamin. Nicotinic acid has been familiar to chemists for about 75 years.
zuki and coworkers first isolated it from plant materials in 1912.
nk ('11) encountered it while attempting to isolate the antineuritic
tor of the vitamin B-complex but since at that time the only biologi-
test for the vitamin B-complex was the cure of polyneuritis he did
recognize its importance in nutrition. Szymauska and Funk ('26)
rrowly missed making a discovery of its significance when they
ured evidence that nicotinic acid exerted a food-sparing and weight-
taining action. From time to time thereafter nicotinic acid was a
ject of investigation by biochemists.

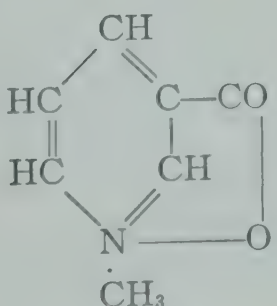
Nicotinic acid has the structural formula



Nicotinic acid



Nicotinamide



Trigonellin
(Methyl betaine of nicotinic acid.)

It melts at 228°-229° C.; it readily sublimes and is soluble in water and alcohol.

A number of investigators (Harris, '37 l.c.; Schmidt and Sy stricker, '38) because of the variable results obtained upon the t ment of pellagrins, especially chronic cases, with nicotinic acid gest that nicotinic acid may not be the only or principal defici in pellagra. In explaining the effects of nicotinic acid or its de tives, Elvehjem suggests the theory that the acid is probably esse in one of the enzymes that transfers oxygen from the blood to cells. Apparently the animal body cannot build the vitamin f the foods but must get it ready made.

Warburg's researches of the role of pyridine compounds in cer biological oxidation-reduction reactions point to the relationship tween some of the vitamins of the B-complex and some enzyme prime importance in metabolism. Warburg et al. ('35) and Schl and Euler ('36) demonstrated that nicotinamide is the active gr in certain coenzymes. The hydrogen transporting coenzyme of Warburg-Christian enzyme system yields on hydrolysis one m cule of nicotinic acid. The amide is evidently an essential constitu of the cozymase molecule. It is regarded as the functional group cozymase which has a fundamentally important part in fermentati glycolysis, and respiration. The discovery of the amide of nicoti acid as an important constituent of living cells is at first sight v surprising and unexpected. It is of interest to note at this point t for several years the diethylamide of nicotinic acid has been wid employed under the name of "coramine" as a heart stimulant in ca of collapse. Kuhn and Vetter ('35) isolated nicotinic acid amide fr heart muscle and it now appears that Warburg believed the coenzy to be present in heart muscle.

As a portion of the cozymase molecule, nicotinic acid amide comes an essential link in the chain of catalysts involved in cert biologic oxidations and reductions.

It appears then that nicotinic acid is either the pellagra-preventi vitamin, or a provitamin, or that it is only one, two, or more su stances essential for the prevention of this deficiency disease. On t other hand, it may be conjugated with other substances in the bo into a more complex compound that is essential to counteract pellag This possibility is suggested by the fact that Warburg's enzym diphosphonucleotide and triphosphonucleotide, each contains a mo cule of nicotinic acid amide.

Dietary Treatment of Human Pellagra.—Sebrell ('38) points o that the essential points in the dietetic treatment of pellagra are: I clusion in the diet of at least 1 quart of milk daily; a caloric intake 3000 to 4000 calories per day, to be supplied in addition to milk a

n meats, liver, and other foods rich in the pellagra-preventive vita-
n; not less than 30 grams and up to 200 grams or more daily of pure
ed powdered yeast, depending on the severity of the attack. Liver
racts administered either orally or parenterally.

It should be emphasized that the administration of nicotinic acid
a supplement to the diet in pellagrous regions, while it may well
vent this disease, is not the most desirable way to meet the prob-
n of pellagra prevention. The pellagra-producing diet, in any coun-
, is deficient in other essential nutrients, and the prevention of
s disease will not necessarily, nor indeed generally, insure a satis-
tory standard of health. As emphasized by Sebrell ('37, l.c.), gar-
ning for the production of home grown foods which provide not
y the indispensable nicotinic acid, but other necessary food factors,
to be strongly recommended. Permanent control can be obtained
y by changing the dietary habits of the people.

Sebrell ('38, l.c.) has fully reviewed the literature on the treatment
this disease, and presents a table of foods having pellagra-preventive
ue and of foods which have little or no value in this respect.

REFERENCES

LING and ARNOLD (1923). J. Am. Med. Assoc., *80*, 365.
MBON (1910). J. Trop. Med. Hyg., *13*, 271, 287, 305, 319.
LDBERGER, WARING and WILLETS (1915). U. S. Pub. Health Reports, *30*,
3117.
LDBERGER and WHEELER (1915a). U. S. Pub. Health Reports, *30*, 3336.
LDBERGER (1922). J. Am. Med. Assoc., *78*, 1676.
LSON (1921). J. Hyg., *20*, 1.
LAND (1920). Lancet, *i*, 947.
LDBERGER (1916). U. S. Pub. Health Reports, *31*, 3159.
NCER (1916). Am. J. Vet. Med., *11*, 325.
ITTENDEN and UNDERHILL (1917). Am. J. Physiol., *44*, 13.
ITTENDEN (1907). "The Nutrition of Man." F. A. Stokes Co., New
York.
DERHILL and MENDEL (1925). U. S. Pub. Health Reports, *40*, 1087.
DERHILL and MENDEL (1928). Am. J. Physiol., *83*, 589.
RY (1920). (Quoted by Wheeler, Goldberger and Blackstock, U. S.
Pub. Health Reports, *37*, 1063, 1922.)
HEELER, GOLDBERGER and BLACKSTOCK (1922). U. S. Pub. Health Re-
ports, *37*, 1063.
LDBERGER and LILLIE (1926). U. S. Pub. Health Reports, *41*, 1025.
IGHT (1932). (Cited. Ann. Rev. Biochem., *1*, 347.)
ITH (1932). "Oral Spirochetes and Related Organisms in Fuso-Spiro-
hetal Disease." Williams & Wilkins Co., Baltimore.
LER and RHOADS (1935). J. Exp. Med., *61*, 173.
ITH, PERSONS and HARVEY (1937). J. Nutr., *14*, 373.
ITH and HENDRICK (1926). U. S. Pub. Health Reports, *42*, 201.

Goldberger, Wheeler, Lillie and Rogers (1926). U. S. Pub. Hea
Reports, *41*, 297.
Smith and Ruffin (1937). Arch. Internal Med., *59*, 631.
Spies (1935). Arch. Internal Med., *56*, 920.
Boas (1927). Biochem. J., *21*, 712.
György (1934). Nature, *133*, 498.
György (1937). Proc. Soc. Exper. Biol. & Med., *37*, 313.
Birch, György and Harris (1935). Biochem. J., *29*, 2830.
György (1935). Biochem. J., *29*, 741.
Kinnersley, O'Brien, Peters and Reader (1933). Biochem. J., *27*, 225.
Dann and Subarrow (1938). J. Nutr., *16*, 183.
Edgar and Macrae (1937). Biochem. J., *31*, 886.
Chick and Copping (1930). Biochem. J., *24*, 1764.
Dann (1936). J. Nutr., *11*, 451.
György (1938a). J. Nutr., *16*, 69.
Aykroyd and Kushman (1936). Ind. J. Med. Res., *24*, 411.
Birch (1938). J. Biol. Chem., *124*, 775.
Fouts, Helmer, Lepkovsky and Jukes (1938). J. Nutr., *16*, 197.
Birch and György (1936). Biochem. J., *30*, 304.
Keresztesy and Stevens (1938a). Proc. Soc. Exp. Biol. & Med., *38*, 64.
György (1938b). J. Am. Chem. Soc., *60*, 983.
Lepkovsky (1938). Science, *89*, 169.
Keresztesy and Stevens (1938b). J. Am. Chem. Soc., *60*, 1267.
Ringrose, Norris and Heuser (1931). Poultry Sci., *10*, 166.
Kline, Keenan, Elvehjem and Hart (1932). J. Biol. Chem., *99*, 295.
Ringrose and Norris (1936). J. Nutr., *12*, 535, 551.
Lepkovsky and Jukes (1936). J. Biol. Chem., *114*, 109; *115*, 557.
Jukes (1937). J. Biol. Chem., *117*, 11.
Fouts, Lepkovsky, Helmer and Jukes (1936). Proc. Soc. Exp. Biol. &
Med., *35*, 245.
Koehn and Elvehjem (1936). J. Nutr., *11*, 67.
Sebrell, Hunt and Onstott (1937). U. S. Pub. Health Reports, *52*, 235
Ruffin, Persons, Harvey and Smith (1937). J. Clin. Investig., *16*, 663.
Sydenstricker and Thomas (1937). South. Med. J., *30*, 14.
Elvehjem (1937). J. Am. Chem. Soc., *59*, 1767.
Elvehjem, Madden, Strong and Woolley (1938). J. Biol. Chem., 123
137.
Smith, Ruffin and Smith (1937). J. Am. Med. Assoc., *109*, 2054.
Fouts, Helmer, Lepkovsky and Jukes (1937). Proc. Soc. Exper. Biol
& Med., *37*, 405.
Harris (1937). Chem. and Ind., *56*, 1134.
Spies, Cooper and Blankenhorn (1938). J. Am. Med. Assoc., *110*, 622
Spies, Bean and Stone (1938). J. Am. Med. Assoc., *111*, 584.
Bogart (1938). J. Am. Med. Assoc., *111*, 613.
Chick, Macrae, Martin and Martin (1938). Biochem. J., *32*, 10.
Woolley, Strong, Madden and Elvehjem (1938). J. Biol. Chem., *124*
715.
Elvehjem and Frost (1937). J. Biol. Chem., *119*, xxxiv; *121*, 255.

YÖRGY (1938c). Proc. Soc. Exper. Biol. & Med., *37*, 732.
ULER and MALMBERG (1936). Biochem. Z., *284*, 455.
UNK and FUNK (1937). J. Biol. Chem., *119*, xxxv.
NIGHT (1937). Biochem. J., *31*, 731.
UZUKI, SHIMAMURA and ODAKE (1912). Biochem. Ztschr., *43*, 89.
UNK (1911). J. Physiol., *43*, 395.
ZYMAUSKA and FUNK (1926). Chem. Zelle u. Gewebe, *13*, 44.
CHMIDT and SYDENSTRICKER (1938). J. Am. Med. Assoc., *110*, 2065.
ARBURG, CHRISTIAN and GRIESE (1935). Biochem. Ztschr., *282*, 157.
CHLENK and EULER (1936). Naturwissensch., *24*, 794.
UHN and VETTER (1935). Ber. deutsch. chem. Gesellsch., *68*, 2374.
EBRELL (1938). J. Am. Med. Assoc., *110*, 1665.

CHAPTER

XXII

Other Vitamin Factors

IN PREVIOUS chapters the nature and functions of the bet known and well-recognized vitamins have been discussed (exc vitamin E, to be considered subsequently). In this chapter the pr cipal evidence which has been presented for a number of dieta factors that appear to be essential for the normal development different species will be considered.

Vitamin B_3 is a term employed by Carter and O'Brien ('35) designate a highly thermostable factor from yeast believed necessa for the pigeon. A number of investigators (Williams and Waterma '27, '28; Carter et al., '30; Eddy, Gurin and Keresztesy, '30) demo strated that when pigeons were fed polished rice supplemented wi vitamin B_1 (thiamin) in the form of fuller's earth adsorbate or oth sources of B_1 (thiamin), the birds gained weight at first, then su fered a decline, suggesting deprivation of a stored factor or factor The food intake was inadequate, especially in respect to protein. Th inadequacy could be remedied by addition of casein or wheat glute It was postulated that the extent of weight recovery varied with th degree of depletion of the store of B_3. The remarkable advances sin this work was published, which have revealed the nutritive signi cance of riboflavin, nicotinic acid, the filtrate factor, etc., make it u profitable to attempt an analysis of the nature of the hypothetical B Reader ('29) used the term B_3 to designate a second heat-labile fact in yeast.

Vitamin B_4 and the Antiparalysis Factor.—Vitamin B_4 is th term first applied by Reader ('30) to a heat- and alkali-labile fact in yeast essential for weight maintenance in rats in addition to (thiamin). The symptoms caused by its deficiency, closely resemblin pink disease in man, were described as muscular weakness, spast

t, swollen red paws, and a tendency to sit in a hunched position. om what has been said of the effects of deficiency of riboflavin d vitamin B_6, it appears that the condition encountered by Reader resented a multiple deficiency state involving these nutrients. Other estigators (O'Brien, '34; Harris, '35; Kinnersley et al., '35) believe t vitamin B_4 deficiency is a chronic phase of thiamin deficiency. owever, Kline et al. ('36) and Elvehjem and Arnold ('36) con- med and extended Reader's original observation with rats. Subse- ently Keenan et al. ('33) employed the term vitamin B_4 to desig- e a factor found in liver and in acid charcoal extracts of yeast epared by the method of Kinnersley et al. ('33). Using a basal diet mposed of casein, dextrin, salts, yeast, and cod liver oil, they ob- ved a leg paralysis, a condition which has been described by some estigators as "leg weakness," and also brain degeneration in chicks. ey found that this antiparalysis factor cannot be extracted from g liver by ether and that it remains in the extracted residue. It soluble in water and is destroyed by autoclaving at the natural H of liver. They regard it as probably identical with Reader's B_4. ader's B_4 was absorbed on acid charcoal, whereas the B_4 factor the Wisconsin group occurred in the acid charcoal extracts. There- e these two B_4 factors are not necessarily identical. However the ture of the factors designated as vitamin B_4 is still obscure.

Keenan and associates regard the paralysis in chicks to be identical th the nutritional encephalomalacia in birds described by Pappen- imer and Goettsch ('31). In nutritional encephalomalacia there are tensive lesions of the cerebellum and occasionally of the cerebrum. enan states that in their chicks, gross and microscopic changes oc- rred in most instances in the cerebrum and in a few cases in the ebellum. Hogan and Boucher ('32) have made observations similar those of Keenan and associates.

Vitamin B_5 is another member of the swarm of factors which erged from the earlier attempts to interpret the cause of weight s in pigeons fed a polished rice diet. It is a slightly water-soluble, t- and alkali-stable factor and is found in whole wheat and yeast arter et al., '30 l.c.). More searching recent investigations have vitamin B_5 isolated in the literature as an entity of ghost-like uity.

Factor Y.—It is impossible to accurately appraise certain meri- ious investigations which were clearly among the first to indicate t the "B-complex" is of multiple nature. Among such studies are se of Chick and Copping ('30) who showed that dermatitis eloped in rats receiving a diet of purified substances supplemented h thiamin and with riboflavin, the former as Peter's purified ncentrate of yeast and the latter as a preparation from egg white

representing the "B_2" group of nutrients. They found that t dermatitis was cured by autoclaved yeast and yeast extracts. Th investigators employed the term "Y factor" to designate the dermati preventing substance. This nutrient is relatively stable to heat, lig and alkali. It is present in autoclaved yeast, aqueous extracts of yea green vegetables, egg yolk, and beef liver. It is almost entirely abse from egg white, wheat germ, and lean beef. These researches we carried out before the emergence of nicotinic acid, factor W, the filtra factor, etc., as nutrients; therefore further investigations will be nec sary for an interpretation of these studies.

Vitamin H.—The term vitamin H has been employed to deno three entirely different factors. György ('35) uses the term to designa the hypothetical nutrient which prevents the so-called "egg whi injury." Since György found vitamin H to be water-soluble only upo enzymatic hydrolysis of the foodstuffs containing it, it is not really part of the vitamin B complex. This investigator ('37) has recent reported that vitamin H appears to be an ampholyte and in particul an acidic amino acid.

Booher ('37) applied the term vitamin H to designate a heat-stab and alkali-labile factor essential, in addition to thiamin and flavin, fo growth and for the prevention of erythrodemic dermatosis in rats. appears to be similar to what is now generally known as vitamin B McCay, Bing and Dilley ('28) studied the problem of rearing youn trout on diets of purified foodstuffs, and found that they could not b reared unless some fresh meat was provided. The unknown facto provided by the fresh meat they called factor H. The substance heat-labile. It appears to be present in all kinds of fresh animal tissue McCay ('37) has reviewed the literature on this subject.

Factor W, or the alcohol-ether precipitate factor, is a nutrient of th G (B_2) complex which apparently is required by the rat for riboflavi to exert its full action. During their investigations of the vitamin complex, Elvehjem and coworkers ('36) discovered that in additio to thiamin, B_4, B_6, riboflavin, and the filtrate factor, the rat require still another nutrient which they term factor W. This factor, precipit able from liver extracts by mixtures of alcohol and ether, is extremel active in promoting growth in rats, but has no effect upon the der matitis of chicks. Although not adsorbed by fuller's earth, there is som doubt whether it is distinct from vitamin B_6 (Halliday and Evans '37). Factor W is strongly stable to acid and alkali in the cold an moderately so at higher temperatures. Being heat-labile, it belongs t the group of thermolabile B factors.

In a recent publication Elvehjem and Frost ('37) present evidenc suggesting a relation of this factor to the pyridine nucleotides. The obtained definite growth responses with their diet on the addition o

denine nucleotides, nicotinic acid amide, and riboflavin. Adenine ucleotides alone produced an immediate though not continuous re- ponse. Nicotinic acid amide alone gave at first a very poor growth esponse, but after about two weeks there was a definite and sustained ncrease in rate of growth. The combination of these two supplements aused a more immediate and continuous response. These results point o the significance of adenine nucleotides as fundamental nutrients.

That some further elusive nutrient for the rat remains to be identi- ed is suggested by the finding of Emerson and Evans ('38) who upplemented their diet No. 805, composed of extracted casein, sucrose, rd, cod liver oil, and a salt mixture, with thiamin, riboflavin, B_6 s a molasses eluate, and yeast nucleic acid. The latter contains adenine ucleotide. These animals failed to show a normal growth response, ut such response was secured by provision of a supplement of a ultiply adsorbed liver filtrate. Elvehjem and Frost (l.c.) secured good rowth response with a daily supplement of 1.0 mg. of adenine nucleo- de. Emerson and Evans ('38, l.c.) administered the equivalent of 1.0 g. of adenine nucleotide in the form of yeast nucleic acid, yet they id not get an equivalent response. One possible explanation for the fference in their results is that adenine nucleotide in yeast nucleic id does not become available in the animal through digestion and sorption; and that they were providing this nucleotide in the form their multiply adsorbed liver filtrate. It is of interest, in the light the possible nutritive significance of adenine (or its nucleotide), to te that Buell and Perkins ('28) showed that adenine nucleotide is a rmal constituent of the blood of various species, and occurs there surprisingly large amounts.

The chemical behavior of factor W towards barium and mercury lts and to the complementary action to flavin points to a similarity the coenzymes. At present, however, it is difficult to evaluate the sults on factor W owing to the non-synthetic nature of the diet used, r vitamins B_4 and B_6 and small amounts of the filtrate factor were ovided in the form of white corn in this experiment.

Factor U.—Stokstad and Manning ('38) observed that yeast and heat middlings had a growth-stimulating effect on the chick other an that produced by the filtrate factor or riboflavin. This appears to distinct from any of the growth substances heretofore described as dispensable for the chick. The basal ration used in this investigation, mposed of polished rice, water-washed fish meal, soy bean oil, sardine l, thiamin, rice, bran filtrate, whey adsorbate, salt mixture, and ound limestone, furnished adequate amounts of thiamin, riboflavin, trate factor, vitamins A, D, K, and the gizzard factor. Addition of cotinic acid had no effect on growth.

This factor is abundant in yeast, middlings, wheat bran, and alfalfa.

It is present to a lesser extent in corn and in very small amounts polished rice. There is none in molasses.

The substance is insoluble in ether, acetone, or isopropanol. It soluble in water and in mixtures of water and methanol. It is adsorb on fuller's earth and on activated charcoal and can be eluted by a mi ture of pyridine, methanol, and water. Autoclaving yeast does n destroy its activity, whereas autoclaving alfalfa does destroy it.

This growth factor is not identical to vitamin B_4; no paralysis produced by a deficiency of it. Neither is it vitamin B_6 as is show by its distribution. B_6 is abundant in yellow-corn, whereas even lar amounts (55%) of yellow corn do not furnish protection to chic deprived of the substance. Molasses, a rich source of B_6, is entire lacking in the new growth stimulant.

Until a chemical name can be assigned, the authors have called tentatively, factor U.

Vitamin K, The Antihemorrhagic Factor.—The observations Dam ('29, '30), Horvath ('30), McFarlane et al. ('31), Holst an Halbrook ('33), Dam and Schønheyder ('34), and Almquist and Stok stad ('35) have demonstrated the necessity in the nutrition of chick ducks, and geese, of a fat-soluble factor distinct from vitamins A, D and E. Deficiency of this substance in the chick was brought about b feeding cereals and even by a ration containing 15 per cent yeas This deficiency disease which resembles scurvy is characterized by sub cutaneous, intramuscular, and abdominal hemorrhages, and by anemia Because of the prolonged coagulation time of the blood in the animal deficient in this substance, the Danish investigators applied the term vitamin K (Koagulations-Vitamin) for this factor. Dam and asso ciates ('36) and Schønheyder ('36) have shown that the failure of th blood to clot properly appears to be due to a decrease in the prothrom bin in avitaminosis K.

Almquist and Stokstad ('36a) have demonstrated the transfer o the antihemorrhagic factor from hen to chick through the egg yolk They noted indications of a certain degree of synthesis of this facto in the intestinal tract of chicks receiving a diet free from vitamin K The vitamin appears to be synthesized by bacterial action, since it amount tends to increase in feedstuffs subjected to such action. Dan et al. ('37) also used dogs, rats, guinea pigs, rabbits, and pigs to de termine their need for the antihemorrhagic substance. They studie the changes in the clotting time of blood from these animals followin administration of vitamin K under experimental conditions. Howeve the results in mammals are not conclusive. It may be that certai species have no physiologic need for this factor or that these animal are either able to synthesize the antihemorrhagic substance or procur it from synthesis of the vitamin by intestinal bacteria. No evidence a

o its relation to hemorrhagic diseases in humans has yet been pre-ented.

Dam and his associates further studied the possible function of his factor in man. No beneficial effects were obtained from use of itamin K in a case of hemophilia. More extensive investigation may urnish data of greater significance as to the role of this factor in the lotting of mammalian blood.

Hog liver fat, hemp seed, soy bean oil, and rice bran are good ources of this nutrient. It is present in vegetables, particularly the afy varieties and to a lesser degree in cereals. The fats from rye, orn, rice, sunflower, cod liver, and wheat germ oil, orange oil, fresh arrots, lemon juice, liver extract, egg yolk, egg white, and alfalfa ash ere inactive as respects vitamin K activity (Almquist and Stok-ad, '36b).

The antihemorrhagic factor occurs in the easily soluble non-sterol raction of the unsaponifiable matter; its activity, however, seems to be narkedly increased by the presence of the fatty acid fraction (Dam t al., '36, l.c.). It is a colorless, unsaturated compound, unstable to lcoholic alkalis even in the absence of air. It is fairly stable to light, eat, and standing, and it easily distills *in vacuo*.

Recent experiments of Klose, Almquist and Mecchi ('38) indicated hat the activity of the vitamin was destroyed by oxidizing agents, rong acids, aluminum chloride and a number of reagents which have he ability to add across an ethylene linkage. It was not appreciably ffected by reducing agents nor by reagents which react with alcoholic, arbonyl or phenolic groups. These findings show that there is no lcoholic or carbonyl group in the vitamin molecule and that its prop-rties are consistent with those of a complex unsaturated hydrocarbon. cetone is a good solvent for the vitamin and acetone or alcohol ex-racts are more active than the food without extraction, indicating that xtraction is incomplete in the alimentary tract.

Almquist ('36) has described a method for obtaining concentrates f vitamin K by extraction of foodstuffs with hexane. Distillation of his concentrate in high vacuum gives a yellow viscous oil which is a ery potent source of vitamin K. He ('37) has also described the prepa-ation of vitamin K in crystalline form. The crystalline material is ree from nitrogen, sulphur, and phosphorus but there is slight evi-ence that the molecule contains one or more benzene nuclei.

The Anti-Gizzard-Erosion Factor.—In avitaminosis K erosions of he lining of the gizzard were frequently observed. Early in these udies it was accepted by the investigators in this field as a part of the yndrome of vitamin K deficiency.

Almquist and Stokstad ('36, l.c.) found that erosion of the gizzard vas cured by a saponifiable fraction of the hexane extract of alfalfa

or dried kale. Their anti-gizzard-erosion factor is fat-soluble and a pears to be thermolabile and readily destroyed by alkalies. They su ceeded in concentrating it to some extent by freezing out the inacti material from an hexane extract of wheat bran oil. This substance do not seem to be necessary for growth.

In recent experiments Almquist and Mecchi ('38) found th cholic acid is an effective dietary supplement in the cure or preventi of erosions of the gizzard lining. Dehydrocholic acid is almost equal effective, while deoxycholic acid is comparatively ineffective.

Cholic acid is an integral part of the gizzard lining and a deficien of bile or of cholic acid in the chick leads to erosions of the lining.

In their studies of B_4 deficiency in chicks, Bird et al. ('36) an Kline and associates ('36) constantly noted gizzard erosion. The found dried lung tissue to be the most potent source of the gizzard fa tor. Liver and kidneys also contained it. Among the cereals, oats is th best source; wheat middlings and bran compare favorably with oat wheat and corn are fair sources, and alfalfa is ineffective. In thei experience this factor could not be extracted with alcohol or ethe Their anti-gizzard-erosion factor is labile to both dry heat and auto claving at 120° C.

Thus a discrepancy exists between the work of Bird and associate ('36, l.c.) and those of Almquist et al. ('36, l.c.) in their findings a to the solubility of the anti-gizzard-erosion factor and as to its dis tribution. The former group of investigators believe that the factor i specific and that it is a water-soluble substance, while Almquist and coworkers present equally convincing data that it is a fat-soluble factor.

The Wisconsin group of investigators (Bird and Oleson, '38) were led by the observation on the richness of the anti-gizzard-erosion fac tor in pork lung to investigate the connective tissue of lung, and othe types of connective tissue. They found that feeding 10 per cent o cartilage afforded excellent protection, but doubtful results were se cured by feeding beef tendon and yellow elastic tissue. Still more sur prising is their finding that protection against gizzard erosion follows feeding of 3 to 5 per cent of chondroitin, an amino polysaccharide con jugated with sulfuric acid. Glucosamine, glucuronic acid, and galac tose, all components or derivatives of chondroitin, were ineffective.

Further investigation will determine whether the anti-gizzard erosion factor is a specific one and whether more than one substance i involved in the maintenance of the healthy condition of the lining o the gizzard in chicks.

The "Grass Juice" Factor.—Elvehjem, Hart, Jackson and Wecke ('34), Stirn, Elvehjem and Hart ('35), and Kohler, Elvehjem and Hart ('37) have shown by feeding studies that green grass and grass

uice contain one or more substances which greatly enhance a diet upplemented with all known vitamins. The factor is abundant in ummer milk and is present in winter milk in regions where feeding ith silage is practical. The amount is so small in winter milk as to e the limiting factor in the growth of rats fed such milk, even when he known inorganic deficiencies (iron, copper, and manganese) of uch milk are supplied. The following table from the paper by Kohler t al. ('37, l.c.) illustrates the distribution of this factor:

THE EFFECT OF VARIOUS SUPPLEMENTS AS ADDITIONS TO MINERALIZED WINTER MILK

(Male rats only)

SUPPLEMENT	LEVEL FED	GAIN OVER CONTROLS	GAIN IN WEIGHT
	per day	*gm. per day*	*gm. per day*
od liver oil	0.1 gm.	0.08	...
range juice	1.0 cc.	−0.52	...
efatted wheat germ	1.0 gm.	0.27	...
rewer's yeast	0.1 gm.	0.14	...
rewer's yeast	0.5 gm.	0.49	...
ried hog's brain	0.25 gm.	0.55	...
$_2$ concentrate	0.5 gm. liver extract	0.55	...
resh lawn grass	3.0 gm.	1.68	3.91
rass juice	0.5 cc.	0.91	...
rass juice	3.0 cc.	1.69	4.48
ried oat grass No. 180	0.6 gm.	1.36	3.98
ried oat grass No. 223	0.6 gm.	0.55	3.17
iver extract sample 1	0.2 gm.	1.38	...
iver extract sample 1	0.5 gm.	1.23	3.40
iver extract sample 2	0.5 gm.	0.67	...
ice bran	0.2 gm.	1.29	3.77

These investigators point out that the grass factor is not vitamins A, D, C, thiamin, B_2, B_4, B_6, all of which were eliminated by their ests, in which inferior growth responses were secured when mineralzed winter milk was supplemented with cod liver oil, orange juice, rewer's yeast, dried brain, and defatted wheat germ. Summer milks re relatively rich in the grass factor.

Hogan and Johnson ('36) found that when guinea pigs or rabbits ere fed a diet consisting of ground oats, skim milk powder, wheat erm oil, cod liver oil, NaCl, $CaCO_3$, the animals could grow but not eproduce. Although it is not stated whether ascorbic acid was proided, undoubtedly it was included; otherwise growth would not have ccurred. When, however, the ration was supplemented with grass

juice, a dilute alcohol extract of the juice of young cereal grasses, o or barley, or an ether extract of dehydrated alfalfa, reproduction came possible. However the two kinds of animals responded son what differently to the alcohol and ether extracts of these plant tissu In case of the alcohol extract alone, advanced pregnancy in the guin pig was rarely observed and there was evidence of abortion. Wh normal litters were cast, the mortality was low but growth was n normal. With the ether extract alone, the animals became pregna but the ration was apparently still inadequate because some of t mothers died after delivering the young. In the ones that survive the young seemed normal at birth, but some were dead and the othe grew slowly for a time and then died. These workers believe that t alcohol extract is a lactation factor and the ether extract is essenti during pregnancy. A combination of the two extracts was much mo effective.

L_1 and L_2 Lactation Factors.—Nakahara and Inukai ('34-35, '3 '36, '36-37, '38) report that a diet consisting of polished rice, fish pr tein, butter, salts, and brewer's yeast or the acid earth adsorbate yeast extract, was adequate for growth, pregnancy, and parturition rats but failed to support lactation. In order to secure normal lactatio two substances, which these workers designate factor L_1 and facto L_2 must be added to this diet. The two factors are different substance since one cannot be substituted for the other. They are equally esse tial, for both together are needed to produce the desired effect.

Factor L_1 is present in beef liver and is obtained from an extract this foodstuff freed from its vitamin B-complex.

Factor L_2 is found in baker's yeast and is obtained from it afte the removal of the B-complex.

Beef liver does not contain L_2 and baker's yeast appears to b deficient in L_1.

Nakahara and associates believe that these factors, which are effe tive in minute doses, are vitamins since animals are entirely depende on the diet for their supply. In addition, they propose that vitamin L and vitamin L_2 together constitute the vitamin L-complex.

Factor L_1 was prepared as an extract of beef liver in 40 per cen alcohol. The extract was adsorbed on acid earth at pH 3 to 4 and th active filtrate was precipitated with barium hydroxide, followed b phosphotungstic acid. Each of these precipitates was an active sourc of factor L_1. They later reported ('36-37, l.c.) that factor L_1 alone di not support adequate lactation until supplemented with factor L This was prepared as described, except that the filtrate from the eart was precipitated successively with phosphotungstic acid and $AgNO_3$ + $Ba(OH)_2$. Both precipitates contained the active factor. The nature o these hypothetical factors is unknown.

The Chick Anti-Encephalomalacia Factor.—Pappenheimer and Goettsch ('31, l.c.) described a syndrome in chicks caused by the eficiency of some nutritional factor. They called this disease, char-cterized by extensive lesions of the cerebellum and occasionally of the erebrum, nutritional encephalomalacia. Pappenheimer and Goettsch ound that the disease can be prevented by the feeding of various oils ich as Wesson (cottonseed) oil, hydrogenated cottonseed oil (Crisco), rn oil or peanut oil, soy bean oil, and olive oil. Lard, on the other and, appears to intensify nutritional encephalomalacia (Goettsch and appenheimer, '36). These investigators have ruled out vitamin E as factor in this condition.

Jungherr and Pappenheimer ('37) present some remarkable illus-ations of the different reactions of various species of birds to their ncephalomalacia diet. Whereas chicks suffer extensive lesions of the rain when fed this diet, ducklings do not develop brain lesions, but stead there is a widespread degeneration of the skeletal muscles. The me diet produces in turkey poults selective necrosis of the smooth uscle of the gizzard wall, unaccompanied by significant lesions in her organs or tissues. Pigeons appear to be refractory under these nditions of nutrition.

Ni ('37) using a diet of the same composition as Pappenheimer and oettsch also produced nutritional encephalomalacia in chicks. How-er, he succeeded in preventing this disease by the addition of Chinese latin to the diet. Gelatin freed from fat was quite as effective as the iginal which contained fatty substances.

Thus the experimental syndrome described under the terms chick ralysis and nutritional encephalomalacia is as extraordinary as is at respecting gizzard erosion or hemorrhagic disease in chicks. As ted elsewhere, Keenan et al. (l.c.) and Hogan and Boucher (l.c.) lieve that the chick paralysis produced by them is identical with e chick encephalomalacia of Pappenheimer and Goettsch ('31, l.c.). et Keenan and associates claim that the curative substance is present defatted tissues and is a water-soluble factor, whereas Pappenheimer d Goettsch correct the deficiency they produce by the feeding of rious oils. Goettsch and Pappenheimer ('36, l.c.) have discussed the fferences of their experimental results and those of Keenan and sociates and conclude that the two groups may not be dealing with e same syndrome.

Muscular Degeneration in Herbivora in its Relation to Nutri-on.—Madsen and associates ('32-33) made many attempts to formu-e a satisfactory diet of purified foodstuffs for herbivorous animals t without complete success. A diet composed of cellulose, cornstarch, sein, sucrose, yeast, lard, salts, cod liver oil, and orange or tomato ice daily, produced in rabbits and guinea pigs a state of malnutri-

tion from which the animals eventually died. There was evidence failing circulation, consisting of intermuscular edema of the legs, creased fluid in body cavities, and congestion and edema of the lur There was frequently a severe hemorrhagic enteritis, often associa with large amounts of blood stained fluid in the lumen of the sm intestine. Fatty degeneration of the liver and kidneys was a frequ finding. The skeletal muscular lesions were similar to hyaline, w or Zenker's degenerative changes. There was also a proliferation fibroblastic tissue which tended to replace the degenerating musc The heart muscle was involved in all animals. Gross alterations co sisted of dilations especially of the right ventricle. When the he was yet normal in shape, the myocardium was usually paler th normal, suggesting degeneration and fibrosis. Histologically, hyali and granular degeneration of the heart muscle fibers was seen, and some areas the fibers were necrotic and in process of absorption. The changes were accompanied by an increase in connective tissue whi often completely replaced portions of the degenerated muscle. Co parable changes occurred in goats fed a diet of similar compositio These heart changes being similar to the ones described by Agdu ('26), they studied the question of cod liver oil injury, but with i conclusive results.

Pappenheimer and Goettsch ('31, l.c., '36) have described nut tional muscular dystrophy in rabbits by restricting them to a diet rolled oats, wheat bran, casein, lard, cod liver oil, NaCl, and CaCO One adult rabbit which successfully completed a pregnancy on th diet, when about one year old, upon the performance of a biopsy fro the thigh muscles, showed necrotic fibers. Her young were scrawny an were dead within 24 hours. Histological sections from their muscl showed that the muscular dystrophy had been transmitted to the youn in prenatal life.

These workers conclude that it is not justifiable at the present tim to speak of this disease as a deficiency state but point out that there some evidence that soy bean oil and cottonseed oil exercise a prote tive effect. Madsen ('36) found that the basal diet supplemented wi the non-saponifiable fraction of cod liver oil, and without added fa produced dystrophy to nearly the same extent as the same diet co taining 6 per cent of lard. Substitution of cottonseed oil for lard cause a high degree of protection. He confirms the finding that cod liv oil causes dystrophy and concludes that the fat relationships studi are not the only factors involved in the production of muscle dystr phy.

Morgulis and Spencer ('36) using the diet of Pappenheimer an Goettsch ('31, l.c.) prevented as well as cured the muscle atroph

supplementing the basal diet with fresh green alfalfa, lettuce, heat germ oil, dry alfalfa, and wheat germ oil or whole wheat germ. hese experimenters believe that there are at least two substances in-lved in the prevention or in the cure of muscle dystrophy, both fac-rs being present in fresh green alfalfa or in whole wheat germ. n the other hand, cold pressed wheat germ oil supplies one of these ctors and lettuce or dry alfalfa furnishes the other.

Drying, extraction with water or alcohol, or ethereal-ferric chlo-de, readily destroys at least one of the substances.

Olcott ('38) has made the plausible suggestion that muscular strophy of Herbivora, produced by the experimental procedures out-ned above, is due to a lack of vitamin E caused by the destructive fect of cod liver oil upon this nutrient. The skeletal muscle lesions in s young rats from females on vitamin E-deficient diets were very milar to those in the nutritional muscular dystrophy of Herbivora.

It will be seen from the foregoing that much experimentation will e necessary in order to finally establish the number and nature of the utrient factors which different species of mammals and birds require. pecific differences in nutritive needs exist to an extent not contem-ated a few years ago. Very little is known about the specific sub-ances which are necessary for the nutrition of the lower forms of ving things. McCay ('38) has reviewed some of the literature on this bject, and Knight ('36) has summarized the present knowledge of acterial nutrition.

REFERENCES

ARTER and O'BRIEN (1935). Biochem. J., *29*, 2746.
ILLIAMS and WATERMAN (1927). Proc. Soc. Exper. Biol. & Med., *25*, 1.
ILLIAMS and WATERMAN (1928). J. Biol. Chem., *78*, 311.
ARTER, KINNERSLEY and PETERS (1930). Biochem. J., *24*, 1832, 1844.
DDY, GURIN and KERESZTESY (1930). J. Biol. Chem., *87*, 729.
EADER (1929). Biochem. J., *23*, 689.
EADER (1930). Biochem. J., *24*, 1827.
BRIEN (1934). Chem. and Ind., *53*, 452.
ARRIS (1935). Ann. Rev. Biochem., *4*, 331.
NNERSLEY, O'BRIEN and PETERS (1935). Biochem. J., *29*, 701.
LINE, ELVEHJEM and HART (1936). Biochem. J., *30*, 780.
LVEHJEM and ARNOLD (1936). Nature, *137*, 109.
ENAN, KLINE, ELVEHJEM, HART and HALPIN (1933). J. Biol. Chem., *103*, 671.
NNERSLEY, O'BRIEN, PETERS and READER (1933). Biochem. J., *27*, 225.
PPENHEIMER and GOETTSCH (1931). J. Exper. Med., *53*, 11.
OGAN and BOUCHER (1932). Mo. Agric. Exp. Sta. Res. Bull. No. 798.

Chick and Copping (1930). Biochem. J., *24*, 1764.
György (1935). "Stoffwechsel und Immunologie der Haut, Handbuch Kinderheilkunde." *10*, 45. Pfaundler-Schlossmann, Berlin.
György (1937). J. Biol. Chem., *119*, lxxix.
Booher (1937). J. Biol. Chem., *119*, 223.
McCay, Bing and Dilley (1928). Science, *67*, 249.
McCay (1937). Ann. Rev. Biochem., *6*, 445.
Elvehjem, Koehn and Oleson (1936). J. Biol. Chem., *115*, 707.
Halliday and Evans (1937). J. Nutr., *14*, 45.
Elvehjem and Frost (1937). J. Biol. Chem., *121*, 255.
Emerson and Evans (1938). Proc. Soc. Exper. Biol. & Med., *38*, 195.
Buell and Perkins (1928). J. Biol. Chem., *76*, 95.
Stokstad and Manning (1938). J. Biol. Chem., *125*, 687.
Dam (1929). Biochem. Ztschr., *215*, 475.
Dam (1930). Biochem. Ztschr., *220*, 158.
Horvath (1930). Am. J. Physiol., *94*, 65.
McFarlane, Graham and Richardson (1931). Biochem. J., *25*, 358.
Holst and Halbrook (1933). Science, *77*, 354.
Dam and Schønheyder (1934). Biochem. J., *28*, 1355.
Almquist and Stokstad (1935). Nature, *136*, 31; J. Biol. Chem., *111*, 1
Dam, Schønheyder and Tage-Hausen (1936). Biochem. J., *30*, 1075.
Schønheyder (1936). Biochem. J., *30*, 890.
Almquist and Stokstad (1936a). J. Nutr., *12*, 329.
Dam, Schønheyder and Lewis (1937). Biochem. J., *31*, 17, 22.
Almquist and Stokstad (1936b). Nature, *137*, 581.
Klose, Almquist and Mecchi (1938). J. Biol. Chem., *125*, 681.
Almquist (1936). J. Biol. Chem., *114*, 241.
Almquist (1937). Nature, *140*, 25.
Almquist and Mecchi (1938). J. Biol. Chem. (in press).
Bird, Kline, Elvehjem, Hart and Halpin (1936). J. Nutr., *12*, 571.
Kline, Bird, Elvehjem and Hart (1936). J. Nutr., *11*, 515.
Bird and Oleson (1938). Proc. Am. Soc. Biol. Chem., xi.
Elvehjem, Hart, Jackson and Weckel (1934). Dairy Sci., *17*, 763.
Stirn, Elvehjem and Hart (1935). Dairy Sci., *18*, 333.
Kohler, Elvehjem and Hart (1937). J. Nutr., *14*, 131.
Hogan and Johnson (1936). Proc. Soc. Exper. Biol. & Med., *35*, 217.
Nakahara and Inukai (1934-35). Nutr. Absts. & Rev., *4*, 133; (1935-3
Nutr. Absts. & Rev., *5*, 1046; (1936-37). Nutr. Absts. & Rev., *6*, 1
(1938). Science, *87*, 372.
Goettsch and Pappenheimer (1936). J. Biol. Chem., *114*, 673.
Jungherr and Pappenheimer (1937). Proc. Soc. Exper. Biol. & Med.,
520.
Ni (1937). Chinese J. Physiol., *12*, 281.
Madsen, McCay and Maynard (1932-1933). Proc. Soc. Exper. Biol.
Med., *30*, 1434.
Agduhr (1926). Acta Paediat., *5*, 319.
Pappenheimer and Goettsch (1936). Proc. Soc. Exper. Biol. & Med.,
522.

SEN (1936). J. Nutr., *11*, 471.
GULIS and SPENCER (1936). J. Nutr., *11*, 573.
OTT (1938). J. Nutr., *15*, 221.
CAY (1938). J. Am. Med. Assoc., *110*, 1441.
GHT (1936). Bacterial Nutr. Rep. No. 210, Med. Research Council, ondon

CHAPTER

XXIII

Vitamin E

AN ACCOUNT of the discovery of vitamin E and the role it pla in nutrition begins in the year 1920 when Mattill and Conklin ('2 in studies of the nutritive qualities of milk, found that rats reared cow's milk were incapable of raising young although the milk was su plemented with iron, probably containing copper, and a small amou of yeast. Two years later Evans and Bishop had made sufficient progr in their studies on the relation between nutrition and fertility announce the existence of a dietary factor essential for reproducti (Evans and Bishop, '22). This discovery was followed by a compr hensive investigation of the "reproductive vitamin" in which Evans a associates and Mattill and coworkers contributed a large amount of o present information. The evidence for a dietary factor, apparently no essential for growth but required for reproduction, tended to broad the concept of the role of diet in all aspects of life. At present t chemical investigations of this group of nutrients appear to ha reached the climax of painstaking and remarkable achievement their isolation, identification, and synthesis. The physiological a therapeutic studies have made considerable progress. Now that pu materials will soon be available we may expect to witness rapid a vances in such investigations. Excellent reviews of the subject ha been written by Evans ('32) and Mattill ('38).

Vitamin E and Antioxidants.—For several years there was mu confusion among different investigators of vitamin E owing to peculiar relation to oxidative influences. Under certain conditions, f example, it was stable to high temperatures (up to 250° C.), but the presence of rancid fat it was rapidly destroyed, even at room te perature. A clue to the nature of this extraordinary behavior was fu nished by McCollum and associates (Simmonds, Becker and McCollu

27) and Jones ('27) who found that the inactivating effect of ferrous ulfate on vitamin A could be prevented by means of wheat germ oil, rich source of E. Simultaneously Mattill ('27) showed that diets vhich caused sterility were subject to rapid oxidation and that wheat erm oil was efficacious in preventing such oxidative changes. Some ime later Mattill and Crawford ('30) adduced evidence that the pro- ective property of wheat germ oil was due to the presence of hydroxy roups which inhibit oxidation of fatty acids. The term antioxidant vas employed to designate the protective substances in wheat germ il, and later found in lettuce oil and tomato oil (Olcott and Mattill, 31a; Bradway and Mattill, '34).

Olcott and Mattill devoted much effort to the problem of determin- ng whether the antioxidant effect of vitamin E concentrates and the itamin E effect resided in the same or in different compounds. Studies f a concentrate which exhibited the properties of both E and the anti- xidant furnished important information (Olcott and Mattill, '31b). Vhen the concentrate was treated with petroleum ether and 92 per ent methyl alcohol the vitamin E activity was found in the petroleum ther fraction and the antioxidant went into the methyl alcohol solu- ion. Thus a difference in identity was indicated. Later studies sup- orted this belief since acetylation destroyed the antioxygenic proper- es but left the vitamin E activity unimpaired (Olcott and Mattill, 34). Moreover they found antioxidant effects associated with the vita- nin in wheat germ oil and oils from lettuce, tomato, carrot, alfalfa, ottonseed, corn, sesame, palm, soy bean, and peanut. But there were o demonstrable antioxidant effects in yeast, lard, cod liver oil, palm ernel oil, and castor oil. It was concluded, therefore, that the anti- xidant substances, which they now designate as inhibitols, are not itamin E.

Antioxidants in the food are necessary for the preservation of vita- nin E activity. Cummings and Mattill ('31) correlated the susceptibil- y to oxidation of several fats and oils with the reproductive behavior f rats. They showed that oxidation in the fatty constituents of the iet destroys E, that such oxidation is extensive in some common fats nd oils, and that the amount of vitamin E in any diet is largely de- ermined by the balance between anti- and pro-oxygenic substances. hese findings were extended by Bradway and Mattill (l.c.) who howed that the fat-soluble vitamins in tomato, carrot, and wheat are ccompanied by inhibitors which prolong the induction period of utoxidizable fats. Carotene and lycopene are pro-oxygenic, and horten the induction period of autoxidizable fats. Such informa- ion was of great assistance to investigators in protecting vitamin E rom oxidative destruction during its concentration and isola- ion.

Destruction of Vitamin E by Means of Ferric Chloride a Rancid Fats.—The ready destructability of vitamin E by ferric ch ride or rancid fats, when the content of antioxidants is relatively lo has been of practical use in preparing diets without vitamin E activi Waddell and Steenbock ('28) found that E is more readily destroy by ferric chloride, under suitable conditions, than vitamin A. Seve different rations produced the typical sterility in rats after a dil ether solution of ferric chloride had been evaporated upon them. T iron treatment of rations caused the formation of some substan which actively opposed vitamin E. This was called an antivitamin. could be extracted from the treated ration by ether.

As pointed out by Evans ('32, l.c.) the discovery of vitamin E w due in part to the accidental use of slightly rancid lard in rations whi apparently contained all the necessary nutrients. Since lard is usual deficient in antioxidants, the vitamin E of the rations was destroye Butter fat, cod liver oil, and other fats and oils, particularly if th are in incipient rancidity, destroy E; consequently they are sometim included in experimental rations to destroy this factor.

Isolation of Vitamin E.—As early as 1927 Evans and Burr ('2 had made potent concentrates of vitamin E. In their work the no saponifiable matter from wheat germ oil was treated with pentane an methyl alcohol, thus removing most of the sterols and some oily m terial. After removing the carotenoid pigments and some other inacti material, by means of these solvents, they obtained a red oil of hig activity. Olcott and Mattill ('34, l.c.) obtained similar oils from variou sources. High vacuum distillation provided them with a product co siderably more potent than that of Evans and Burr. Drummond an associates ('35) also prepared highly potent concentrates. After th demonstration by Olcott ('35) and by Drummond and coworke (l.c.) that the vitamin contains a hydroxyl group which can be readil esterified, it became possible to consider the isolation of crystallin allophanates. The reaction of cyanic acid with the hydroxyl group alcohols forms allophanic esters which are generally favorable f identification purposes. By this means Evans, Emerson and Emerso ('36) were able to isolate three different allophanates from wheat ger oil concentrates:

1. Allophanate of β-amyrin with a melting point of 250°. Th regenerated alcohol had no vitamin E activity.

2. Allophanate with a melting point of 138°. The regenerate alcohol had some activity.

3. Allophanate with a melting point of 158-160°. The activity the alcohol regenerated from this substance was much higher, 3 m being sufficient to permit an E-deficient rat to bear young. The em pirical formula $C_{29}H_{50}O_2$ was assigned to this alcohol. According t

vans and coworkers it shows a characteristic absorption band at
98 A., $E^{1\text{ per cent}}_{1\text{ cm.}} = 90$ ca. The name *α-tocopherol* was given
it (Tokos: childbirth; phero: to bear; -ol, indicating an alcohol).
Todd, Bergel, Waldmann and Work ('37a) confirmed the findings
Evans and coworkers, and in addition they isolated a crystalline
lophanate, m.p. 70°, which has not been examined biologically.
odd and associates ('37b) have developed an improved procedure
r separating the allophanates of vitamin E concentrates of wheat
rm oil and rice germ oil. By this means an allophanate was obtained
hich they designated as β-tocopheryl allophanate, m.p. 143.5-144.5°.
is optically active and the parent alcohol shows an absorption
aximum at 295 mμ. They succeeded in securing only traces of the
tocopheryl allophanate described by Evans et al. Since the vitamin
activity of their concentrates, yielding principally β-tocopheryl
lophanate, showed full activity in single doses of 5 mg. per rat they
ncluded that β-tocopherol is the principal vitamin E in wheat germ
l. But Emerson's (38a) extensive studies show that oils vary greatly
their content of the different tocopherols, depending on the locality
d on the year of the crop from which they are obtained.

Emerson, Emerson and Evans ('36) have isolated α-tocopherol
om cottonseed oil, which apparently is a good source of the substance,
compared with wheat germ oil, since it contains less non-saponifia-
e material. They have also obtained γ-tocopherol from the same
urce (Emerson, Emerson, Mohammad and Evans, '37). It appears
obable that there is considerable variability in the proportions of
β,- and γ-tocopherols in various natural substances having biological
tivity. It can be definitely stated that they have uneven E potency.
ne α-substance is more active than the β-or γ-forms. It is probable
at all three compounds are isomeric, and therefore have the same
mpirical structure $C_{29}H_{50}O_2$. On the basis of structural considera-
ns, it appears probable that there are more than the three known
copherols which possess the properties of vitamin E. Definitely,
amin E is of multiple nature.

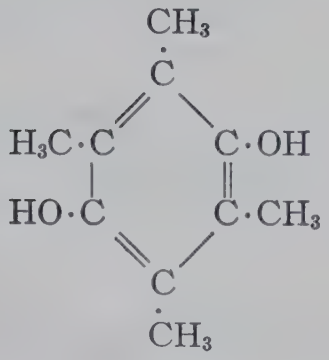
Chemical Structure and Synthesis.—At present (early summer of
38) there is much activity in several different laboratories in studies
the structure and synthesis of vitamin E. Rapid progress is being
ade and it appears that the fundamental chemical investigations will
completed in a short time.

After the empirical formulae of both α- and β-tocopherol had been
ggested by Evans and associates ('36, l.c.), Fernholz ('37) made the
xt advance when he found that α-tocopherol, isolated from cotton-
ed oil, decomposes when heated at 350° and a crystalline sublimate
obtained as well as an oily distillate. The crystalline material, when

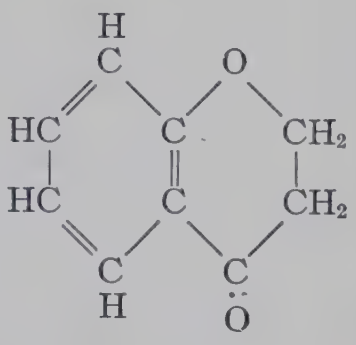
purified, led to a formula $C_{10}H_{14}O_2$. A search of the literature sho that it closely resembled durohydroquinone (i.e. tetramethyl hy quinone), and upon comparison with a pure specimen of this stance was found to be identical with it. Somewhat similar observat were made independently by McArthur and Watson ('37) and Todd, Bergel, Waldmann and Work ('37, l.c.). Since the reduc of a silver nitrate solution with pure α-tocopherol did not result in splitting off of durohydroquinone it appeared that the alkyl por of the α-tocopherol molecule is connected with the aromatic ring means of a carbon bridge (Fernholz, '38). It was necessary, th fore, to abandon the view that the linkage is by means of an et bond.

Various observations, particularly those based on oxidative r tions, indicated a heterocyclic structure. Bergel and coworkers ('3 were led to believe that β-tocopherol is a coumaran derivative si the absorption spectrum and reducing properties of both hepta cylcoumaran and methylcoumaran are very similar to those of p β-tocopherol. Also, Karrer and associates ('38) believed that couma is the derivative of α-tocopherol. However, the observation of Fernh ('38, l.c.), Smith, Ungnade and Prichard ('38), Emerson ('38b), a of John and associates ('38) provide rather convincing evidence t the derivative of α-tocopherol is chroman, instead of coumaran. cording to Fernholz, a lactone is obtained when α-tocopherol is o dized with chromic anhydride in acid solution. This is explainable the basis that α-tocopherol contains a chroman ring, but if it were coumaran ring a beta hydroxy acid should be expected as an oxidati product. There are other evidences which suggest that chroman is basis of vitamin E structure.

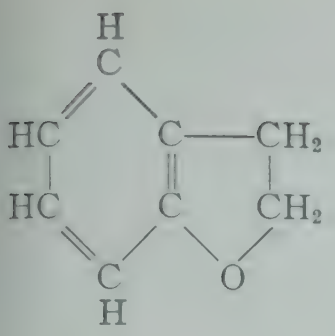
The following formulae indicate the principal compounds un discussion:



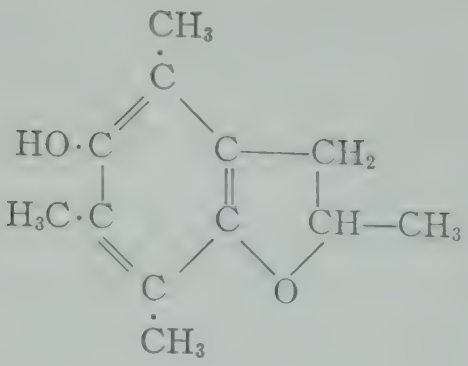
Tetramethyl hydroquinone
(Durohydroquinone)



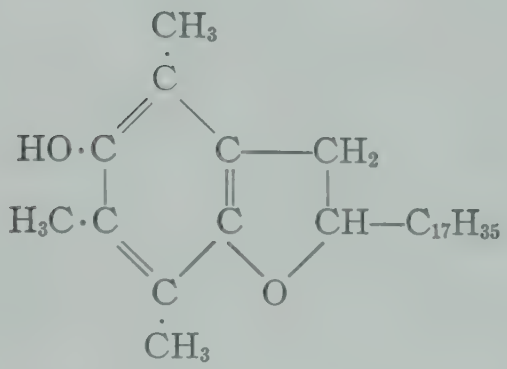
Dihydrobenzopyran
(Chroman)



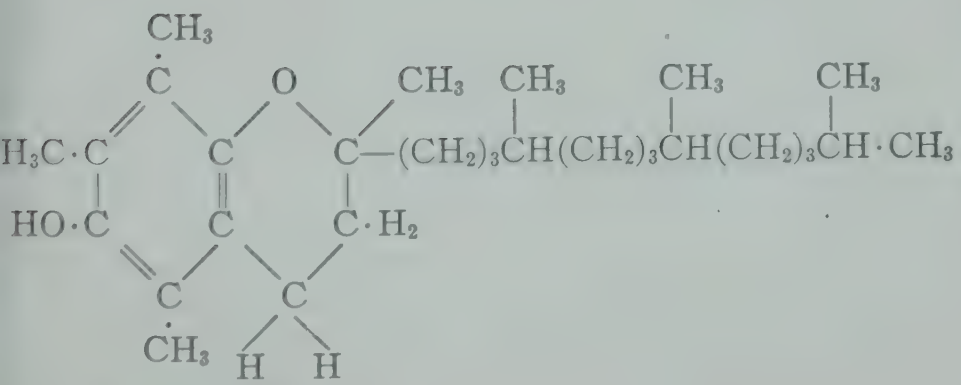
1, 2-dihydrobenzofuran
(Coumaran)



5-hydroxy-2 : 4 : 6 : 7-
tetramethylcoumaran
(Methylcoumaran)



5-hydroxy-4 : 6 : 7-trimethyl-2-n-
heptadecylcoumaran
(Heptadecylcoumaran)



α-Tocopherol (Fernholz)

α-tocopherol was first synthesized by Karrer and associates (using trimethylhydroquinone, phytylbromide and zinc chloride. biological assays were reported. It is probable that the structure t assigned to this substance was in error since they regarded it a coumaran rather than a chroman. Shortly thereafter Smith, Ungn and Prichard (l.c.) reported two different syntheses of α-tocophe one from trimethylhydroquinone and phytylbromide without a c lyst, and also from the hydroquinone and phytadiene. The evide suggested that the products were chromans. Thus the chemical vestigations of vitamin E, slow at first, but gaining momentum w successive findings, have drawn to a successful climax of rapid coveries which again illustrate the method of progress in nutrition.

Vitamin E Activity of Synthetic Compounds.—Important p liminary studies of synthetic compounds have been made by Eva Emerson and Emerson ('38). Vitamin E activity of durohydroquino various of its ethers, and certain cyclic ethers was observed when at high levels. Synthetic α-tocopherol, prepared by Smith, Ungna and Prichard, was active in a single dose of 3 mg. The substance p pared from natural sources has the same potency as the synthe vitamin.

Early Observations on the Nutritional Significance of Vitam E.—Sterility in animals may be caused by any dietary defects wh interfere with growth or which impair health. Thus vitamin A def ency interferes with ovulation, and hence with reproduction. Deficien of an essential amino acid, or of any one of the several vitamins, well as unphysiological relations in the food supply of the indispe able inorganic elements, will lead to failure of normal reproducti Specific instances of such impairment have been given in previ chapters. In vitamin E we meet a nutrient, a deficiency of which i pairs fertility by interference with placental function in the fema and spermatogenesis in the male.

A number of investigators who studied the effects of diets natural foodstuffs restricted as to source, and also diets composed more or less purified food substances, observed low fertility or steril in their animals, but the results could not be interpreted. Evans a Bishop ('22) laid the foundation for all later researches on vitamin by their study of the precise mechanism of the estrus cycle in the r They then turned their attention to the effect of inadequate diets the ovulatory rhythm and on the several steps in the physiology reproduction. Evans ('32, l.c.) stated that "to their surprise they fou that on supposedly complete dietaries, and with both growth a external appearance normal, animals would also exhibit normal est cycles and would breed, ovulate and conceive, yet be unable to expe ence normal gestation through invariable occurrence of fetal deat

systematic study was begun in which vitamins A, "B," C, and D ere supplied in abundance but without remedying the defect. It was und that addition of certain natural foods quickly corrected the normality, and since, in the case of wheat germ or wheat germ oil, e "curative" dose was very small, Evans ('22, l.c.) announced the iscovery of a new, and at that time the 5th vitamin, which he and his ssociates provisionally designated X, and which was later called vita- in E. Confirmatory studies were soon reported by others, notably y Mattill and Stone ('23), Sure ('24), and Mason ('26).

Evans ('32, l.c.) has emphasized that little information concerning tamin E can be gained by merely noting failure to secure young hen the sexes are placed together. It is necessary to observe the oc- rrence of estrus by means of vaginal smears; to ascertain the occur- nce of copulation, by finding of the vaginal plug in the vagina or residual sperm in the vaginal contents; the cessation of estrus cycles determined by daily study of the vaginal smear, up to and including nplantation as detected by the erythrocyte sign (finding of red blood lls in the smear on the 14th to 17th day) which never occurs unless nplantation of the ovum has taken place. It is also necessary to fol- w the course of pregnancy, to determine whether it is normal or hether resorption occurs (which is characteristic of sterility due to ficiency of E), or impairment of the birth mechanism (which occurs some stages of sterility due to vitamin A deficiency). Unfortunately, me investigators have attempted to interpret their studies without bjecting themselves to this rigid and laborious task, and as a conse- uence much of the literature on the various aspects of the vitamin problem is untrustworthy.

The effects of vitamin E deficiency are remarkably different in the o sexes, the only other parallel case known being a deficiency of anganese.

Vitamin E Deficiency in the Female.—Evans ('32, l.c.) states that triking proof that the early phases of the reproductive process do not ed vitamin E is afforded by the resuscitation and in fact the com- ete normality of young in gestations when the curative dose of E not administered until a few hours before implantation of the blas- dermic vesicles—an administration as late as the completion of the h day after copulation. The vesicles are attached to the uterine ucosa some time early in the sixth day." Stigmas of abnormality do t appear until the 8th day. A sufficiently large single dose of E ministered on the 5th day is adequate to enable the female to com- ete a normal gestation. "Though the fetal rather than the maternal sue on the placenta shows selective disorder when E is low or sent, there are indications that the maternal mechanism is abnormal a strange tendency of the uterus in such females to form spontane-

ous nodular deciduomas when pseudopregnancy is provoked by c lation with a vasectonized male." Pseudopregnancy is a remark phenomenon described by Long and Evans ('22). It is manifested delay in the next estrus events, caused by sterile copulation or sti lation of the cervix. These stimuli do not delay or accelerate an pending ovulation, but delay the next following ovulation. The e is immediate but the manifestation is deferred.

The integrity of the implantation site, placental tissues, and f tissues are dependent on vitamin E. The deficiency state is charac ized by many free red blood cells at the site of implantation of ovum, and hemorrhagic areas are seen in the placenta. The sinus are greatly distended. In the fetal tissues the blood islands do not velop normally, and it seems probable that deficient oxygenation, means of this abnormality, may cause impairment of fetal devel ment.

Urner ('31) described the history of 91 Wistar albino rats, depri of vitamin E, which exhibited normal growth, estrus rhythm, breeding behavior. Pregnancy in the entire group of rats was follow by embryonic death and resorption. No first litter fertility was served. Urner found that from the 10th to the 17th day of pregna the embryonic and fetal resorption was more than counterbalan by continued mammary development, so that the body weight c tinued to increase, although at a reduced rate. Gross examination the pregnant uteri (from the 10th day onward) showed a softeni of the enlargements, with blue discoloration, due to blood in amniotic cavity—an indication of abnormal pregnancy. Microsco examination of serial sections of embryos showed apparently norn embryonic development until the 10th day of pregnancy. At this ti there appeared a rarefaction of the mesenchyme, failure of the blo forming tissues, and death of the embryo. Necrosis began at the fe surface of the ectoplacenta by the 13th day, and by the 17th or 1 day the mesometrial portion of the placenta was destroyed and into the lumen of the uterus. The decidua basalis appeared normal a became converted into tissue which, in the normal course, would come the maternal portion of the normal placenta until it was ov taken by degenerative changes on the 15th day. By the end of 21st day of pregnancy the uterus had returned to an almost virgi condition, with the exception of the decidual bodies at the sites recent implantation.

Vitamin E Deficiency in the Male.—Evans described sterility the male rats as occurring at a later date than in their litter m sisters. It can be tested for by mating experiments with females known fertility, but may be predicted by the finding of characteris "sheaves" or clumps of immotile spermatozoa in the fresh ejacul

here is progressive loss of tubular germinal epithelium, and irrepable damage to the basal spermatogonia. Eventually only diminue tubules, devoid of all cells except the Sertoli elements, remain. ch males still possess normal seminal vesicles, prostate, and coagluing glands and although their germinal epithelium has largely dispeared, continue for a considerable time to manifest sex interest and wer to copulate. Later on these sterile males suffer degeneration of e accessory organs and sex interest fades out.

Mason ('33) has reported the most critical study available on the fect of vitamin E deficiency on testicular degeneration. He describes characteristic a liquefaction of the chromatin material of the ermatozoa and spermatids, and later the less mature cells, followed the fusion of injured spermatids and spermatocytes into giant lls.

Even when vitamin E is administered at the time of the earliest gns of impairment of spermatozoa, only a few can be restored to rtility. Before these signs have appeared sterility can be invariably evented by the provision of vitamin E. Evans points out that for e insurance of fertility throughout life in males a higher daily inw of E is essential than for gestation alone: hence the requirement the seminiferous epithelium, in proportion to its mass, is greater an that of the placental or fetal tissues. The latter increase much ster in volume than do the germ cells.

Thus, as pointed out by Mattill ('38, l.c.), "The striking difference response to vitamin E deprivation by male and female animals n be explained, superficially, by the fact that in the male the damage done to a part of the animal's own tissue and may for this reason irreversible in contrast to the reparable damage in the female, which wrought not on her own tissues but on those of the fetus."

Mason ('33, l.c.) has attempted to compare the anatomical effects vitamin E deficiency with those produced by other dietary defects. eficiency of vitamin A, in his experience, caused sloughing of the rminal cells into the lumina of the tubules (attributed by Evans to deficiency). Marked injury to the testes caused by inanition was paired in 3 to 4 weeks when the diet was increased, but injury used by A deficiency required from 5 to 13 weeks for its repair. mbined deficiency of A and E caused less severe damage to the stes than did uncomplicated E deficiency, but deficiency of A creased the time required to cause the changes characteristic of deficiency. He suggests that vitamin E may be particularly essential r the later stages of maturation of the germinal cells. When the rlier stages of differentiation are slowed down by deficiency of A, tamin E deficiency produces less profound histological changes in e germinal elements as a whole.

The Effects of Vitamin E Deficiency on Hatchability of Eg

A principal effect of vitamin E deficiency in hens is said to be reduction in hatchability of the eggs and a decrease in the vitalit chicks that are hatched (Card, Mitchell and Hamilton, '30; Ada tone, '31; Barnum, '35). As shown by Adamstone, the first 3 day development in the egg appear to be normal when the hen has l on an E deficient diet. But the effects of deficiency are precipitous many chick embryos die on the 3rd to 4th days. Hemorrhage various sites are regarded by Adamstone as the immediate cause death. As a rule a "lethal ring" is found completely encircling embryo at the end of the 5th day. The ring occludes the blood ves of the blastoderm and thus asphyxiates the embryo. It is produced extensive cell proliferation in the mesoderm. The nature of this "ri is obscure. It would seem that the mechanism of chick embryo m tality might have a counterpart in the death and resorption of embryos, and perhaps embryos of other Mammalia. The data app to indicate that deficiency of vitamin E is not infrequently a ca of low hatchability and high chick mortality in poultry prod tion.

Effects of Deficiency on Growth.—For several years it appea that vitamin E was specifically a reproductive factor but during t past decade evidence has slowly accumulated which prove that t nutrient is indispensable in general bodily functions. The first in cation of need for it in other processes besides that of reproducti was furnished by Evans ('28) who noted that vitamin E deficien caused a failure in the late stages of growth, "the phase interveni after the attainment of maturity." Several years later Blumberg ('3 by means of a highly refined basal ration, observed an earlier failu in growth and, in addition, marked generalized debilitation. Since t effects were quite striking the observations focused attention on t relation of vitamin E to growth. In the same year Ringsted ('35) ported somewhat similar results, but he did not employ curative p cedures with sources of vitamin E. Emerson and Evans ('37) insist that "The defective growth in experiments involving vitamin E d ficiency occurs only after the fourth month of life." Olcott and Mat ('37) also denied the validity of Blumberg's claims that vitamin or some related factor, is necessary for normal early growth. Howev neither group of workers used basal diets exactly like the one employ by Blumberg. Martin ('37) studied the problem, using Blumber basal diet, and noted that slowing of growth occurs at the 10th we thus essentially confirming the latter's observations. More recen Evans, Emerson and Emerson ('38, l.c.) have stimulated growth female rats by means of pure α-tocopherol fed to the animals for days after they had plateaued in weight on a vitamin E-low diet. Th

is fully established that vitamin E is indispensable for normal owth. Since it is difficult to understand how the growth process can independent of vitamin E during earlier life but require it later, the gical assumption would seem to be that the nutrient is needed for owth irrespective of any particular period in the span of life. Until has been demonstrated that young rats are able to grow normally hough their tissues are completely devoid of vitamin E, the above ims of Evans and associates and of Olcott and Mattill must be garded as without full experimental support.

Paralysis and Vitamin E Deficiency.—A curious type of paralysis associated with marked vitamin E deficiency. As shown by Evans d Burr ('28), young rats from females on a vitamin E-low diet may hibit a marked paralysis, especially of the hind quarters, about 20 ys after birth. Many of the young have difficulty in righting themves when placed on their backs. By the 21st day about 75 per cent the animals are paralyzed in the posterior extremities and in part the body wall musculature. The onset is sudden and some animals e so quickly that there is no loss of weight, showing that starvation not a complicating factor. Others die after a few days, with some gree of emaciation. At the onset of the disability there is an almost nstant exhibition of spasticity of the lower parts of the limbs and flexor spasm resembling the "claw hand." A few, however, show initial flaccid paralysis—a dragging of the hind legs—which is ceeded by the spastic condition. The paralysis is always partial. ose which survive 2 or 3 months are said to appear healthy but y remain partially paralyzed, with atrophic skin, and bilateral dedation over the upper thigh and sacrum. Most remarkably, someat less than one-fifth of such young recover completely. Also, the mals which survive, but remain partially paralyzed, are able to proce young in spite of the paralysis when a vitamin E concentrate is ministered. A single administration of vitamin E, in the form of eat, yellow corn, or lettuce, to such females, after birth of the young, sufficient to protect the latter from paralysis. This suggests that the rage capacity for vitamin E is considerable and that the nutrient ecreted in the milk. But vitamin E does not cure the paralysis after as developed.

Blumberg (l.c.) noted marked stiffness and evidences of muscular urbance in female rats restricted to a vitamin E deficient diet for iods of 30 to 40 weeks. The animals could be cured by the adminision of vitamin E concentrates. Ringsted (l.c.) and Marchesi ('35) observed paresis in older rats on diets low in E. But Olcott and ttill ('35, l.c.) were not able to confirm these observations, claiming t E deficiency was not the cause of this peculiar syndrome. How-r, the evidence undeniably points to the existence of a relationship

between vitamin E and the integrity of muscular or nervous funct concerning locomotion in adult rats. Emerson and Evans ('37, found that females, on a vitamin E-low diet for 19 months, develo an unsteady gait and became partially bald. This has been confirr and amplified by Burr, Brown and Moseley ('37) who restricted yo female rats to a purified diet consisting of casein, 12; sucrose, 8 salt mixture, 3.9; together with supplements of vitamin A, viost nearly fat-free yeast, pure thiamin, and linseed oil. The growth of animals was somewhat below normal, as would be expected. At months of age all the rats (5) became paralyzed, with loss of use their hind limbs. The muscles of the legs and gluteal region were noticeably atrophic. Electrical stimulation showed that sensibility painful stimuli was not affected. Isolated muscles and larger ne trunks of the leg and gluteal region gave normal responses. sensory ganglia of the brain and spinal cord, as well as the poste funiculus of the cord, had a yellow color. The paralysis could not cured by means of vitamin E.

The nature of the paralysis is still obscure. Lipshutz ('36) attributed the condition in young rats to degeneration of nerve tra in the spinal cord. Olcott ('38) has made the important suggesti that some relationship exists between this syndrome and that produc in guinea pigs and other Herbivora on certain purified diets, the called nutritional muscular dystrophy of Herbivora. (See Chap XXII.) He has pointed out that the diets which produce the syndro in Herbivora contain cod liver oil or lard, both autoxidizable su stances which quickly destroy E. The symptoms of paralysis in a mals on the muscle dystrophy-producing diets bear a striking semblance to those on E deficient regimens. This is especially indicat by a comparison of the symptoms noted in rabbits (Morgulis a Spencer, '36) with those seen in adult rats deprived of vitamin (Burr, Brown and Moseley, l.c.). Olcott has concluded that the musc lar lesions which occur in the young of vitamin E deficient rats a very similar to those observed in nutritional muscular dystrophy Herbivora. Since it is believed that the nervous system is not volved in the latter condition, the lesions being primarily musc lar, it is possible that the paralysis of vitamin E deficiency is attrib able to muscular lesions without involvement of the nervous s tem.

Whether the "range paralysis" seen in some species of poultry related to inadequate vitamin E is a subject for investigation. Likew the various other forms of paresis observed in domestic animals, well as man, should be investigated with reference to their possi relationship to disturbances in vitamin E metabolism, or inadequacy this nutrient in the diet.

Vitamin E and Internal Secretions.—It is quite natural that investigators should give considerable attention to the possible relationships between hormones and vitamin E.

As early as 1925 van Wagenen ('25) observed cellular changes in the anterior hypophyses of male rats on an E deficient diet. These changes were further studied by Nelson ('33), who found that there is an increase in the size and number of basophilic cells similar to but less pronounced than that occurring in castrated males. Implantation of these anterior hypophyses into immature female rats showed that they contained more of the gonad-stimulating hormone than did those of normal rats but less than those of castrated rats. In each respect the effect of E deficiency was the same as that of cryptorchidism. The anterior hypophyses of E deficient female rats appeared to be normal. This latter finding is contradicted by Rowland and Singer ('36) who state that hypophyseal suspensions of non-pregnant E deficient rats are only about one-half as potent as those of normal females in promoting ovulation when injected intravenously into the estrus rabbit. They noted that there was a normal amount of gonadotropic substance in the hypophyses of E deficient females, cured by the administration of vitamin E. A third type of response was reported by McQueen-Williams ('34), who transplanted hypophyses of E deficient female rats into immature animals and concluded from the results that there is hyperactivity of the hypophysis in this deficiency, which is somewhat reduced by vitamin E administration. However, the sex-difference noted by Nelson, and others, should be expected on the basis that hypophyseal changes are the direct consequence of gonadal injury which is apparently confined to the male, the injury in females being limited to the placental structures.

Apparently the thyroid also is affected by vitamin E deficiency. Singer ('36) states that the thyroid glands of female rats deprived of E for 12 to 18 months exhibit marked hypoplasia. The condition was not affected by the administration of iodine, but injections of anterior hypophyseal extract caused some improvement. The thyroid became normal when vitamin E was administered. These observations correlate with those of Barrie ('37) who depleted female rats of E until sterility was established. Then the nutrient was administered, but only in very small amounts so that there were only 3 or 4 young per litter in succeeding pregnancies. Although the young appeared to be normal at first they failed to grow and became definitely cretinous. The appearance was characterized by a large head, sparse fur, upturned nose, deformed feet, and fontanelles still widely open. The animals lived 17 to 30 days but whether paralysis occurred was not stated. Singer suggests that E deficiency indirectly affects the thyroid through its influence on the anterior hypophysis.

Vitamin E and the Induction of Tumors.—Since Evans (described the high incidence of deciduomata during pseudopregna in E deficient rats there has been some basis for suspecting that vita E might play a role in tumor formation. However, there is no c indication of such a relationship, although several investigators h studied the question. Davidson's ('35) report that a diet rich in v min E inhibits the development of tar cancer in mice was not c firmed by Haddow and Russell ('37). However, the latter believes t the apparent discrepancy is due to other factors and is not attributa to E. Adamstone ('36), in a continuation of his earlier studies on relation of vitamin E to excessive tissue growths in chick embryos, tentatively concluded that vitamin E sets up some condition favora to excessive cell proliferation. A complicating factor in his experime is the use of ethereal ferric chloride to destroy vitamin E in the rati

The astounding reports of Rowntree and associates ('37) ha stimulated interest in this question. They claim that excessive and p longed feeding of certain types of wheat germ oil, particularly un fined oil obtained by ether extraction, produced a very high incide of peritoneal spindle cell sarcomata in rats on a stock diet. McCollu Becker and Day ('38) have failed to produce a single tumor in r given large doses of cold-press wheat germ oil refluxed with eth The oil was administered for 170 days. Hence it does not seem possib that ether, through the oxidizing effect of peroxides, produces ca cerogenic substances in wheat germ oil. The relation to tumor form tion of substances associated with natural sources of vitamin E requi further investigation.

Vitamin E Assay.—At present there are no satisfactory methods vitamin E assay. This, of course, constitutes a decided handicap studies of this group of nutrients. The best methods available are bas on the determination of the ability of adult E-deficient females to be living young following the oral administration of the test substanc At best the data are crude; moreover, they are obtained at considerab expense. Such procedures have permitted the accumulation of co siderable information concerning vitamin E but they are in need refinement for future investigations.

Bacharach and Allchorne ('38) have devised a curve relating dosa and response to vitamin E. They employ virgin rats depleted of as ascertained by mating tests. The substance to be tested is divid into 10 equal portions and administered daily for 10 days followi positive mating. The results are expressed in terms of "mean fertili dose," the doses that will cause 50 per cent of implanted rats to be litters (Bacharach, '38).

Palmer ('37) has criticized the procedure of administering the te substances in several divided doses, claiming that a single dose is pre

able until more information is obtained concerning the utilization E at different periods of pregnancy. The criticism appears to be lid. Palmer has described in detail a method of assay applicable to heat germ and wheat germ oil. Presumably it could be used for gen- al estimations of E in other foodstuffs. The basal ration is: com- ercial casein, 27; autoclaved tapioca (dextrin), 35; commercial bulk rd, 22; dried brewer's yeast, 10; Hawk and Oser salt mixture, 4; nd cod liver oil, U. S. P., mixed in the ration at weekly intervals, 2.

Distribution in Nature.—Since analytical methods for vitamin E ave not been developed there are no satisfactory data on its distribu- on. On the basis of present evidence, however, it appears to occur many different foodstuffs. The oils from seeds, such as wheat, rice, otton, and corn are rich sources. Leafy vegetables also contain con- derable amounts of the nutrient. Animal tissues and eggs contain it ut they are not generally recognized as potent sources. Butterfat is w in E and it is generally believed to be absent from lard and cod ver oil. Commercial hydrogenated fats are good sources. The occur- nce of antioxidants (inhibitols) in many foodstuffs is important in etermining the vitamin E activity, since in their absence E is rapidly estroyed. Vegetable fats contain them but animal fats do not.

Requirements of Different Species.—Although it seems probable at vitamin E is needed by many different animal species indubitable vidence of its necessity has been established only in the case of rats nd mice. These animals, with reproductive ability impaired by restric- on to E deficient diets, have been restored to a normal condition by oncentrates of vitamin E, i. e., the saponifiable fraction of wheat germ il. This, in addition to the lack of positive effects from the adminis- ation of other known vitamins in generous amounts, constitutes nequivocal evidence of need for vitamin E. In discussing other sub- cts in this chapter we have presumed, on reasonably valid grounds, at E is required by chicks, but most of the studies of such animals ave been based on the use of diets in which E was "destroyed" by eatment with ethereal ferric chloride. Thomas and associates ('38) aim that normal reproduction has been obtained in goats, sheep, nd rabbits restricted to a diet prepared in this manner. The claim at reproductive ability is improved in humans, cattle, and pigs, by eans of wheat germ oil or other rich sources of E, suggest that they quire the nutrient.

Therapeutic Applications of Vitamin E.—There have been veral reports that wheat germ oil or other rich sources of vitamin are efficacious in some cases of sterility. Schioppa ('36) and others ve stated that large doses of wheat germ oil increase the size of obit litters (cf. Thomas et al., *vide supra*). Bay and Vogt-Møller 34), in convincing veterinary studies, have claimed that the injection

of sterilized wheat germ oil into apparently sterile, but other normal, cows and sows permitted reproduction in a majority instances. But many more studies will be necessary to establish value in animal husbandry. The evidence, however, suggests the portance of extending research in this field.

Several clinical reports have appeared which suggest that w germ oil is of value in preventing spontaneous human aborti But much of the data are rather unconvincing because of the lack adequate control. Vogt-Møller ('31, '33, '36) has published the res of several studies in which wheat germ oil treatment appare caused normal pregnancies which otherwise would have termina in abortion. In these studies structural or functional abnormali were ruled out as possible causes of previous reproductive failu Currie ('36) reported a series of 23 women who, between them, l experienced 73 pregnancies, resulting in the birth of 11 living childr five of whom died shortly after birth. He administered 3 mini (0.18 cc.) of wheat germ oil concentrate (5 gm. of oil), and eith "adexolin" (presumably a Ca preparation) or calcium gluconate a calciferol, for periods of about 5 months. Following this, 22 of t women were delivered of full term living children and the pregnan of the remaining one was terminated by abortion. In a later stu (Currie, '37) the use of an unsaponifiable wheat germ oil fracti was followed by 31 full term deliveries in 37 women who had pre ously aborted. Currie states that by means of similar treatment had success in 14 of 15 cases of threatened abortion.

Watson ('36) has administered daily doses of wheat germ oil a series of over 40 women, in each of whom abortion had occurre in 60 to 70 per cent of the cases. By means of larger doses threatene abortions were successfully treated in 13 of 19 cases.

Shute ('35, '36, '37) made the interesting observation that the bloo serum of 70 per cent or more of women aborting spontaneously di plays a characteristic resistance to proteolysis when treated with tryps solutions. He tested the serum of rats of both sexes, which had bee restricted to an E deficient diet, and found it resistant to trypt digestion. From his description of rough coat and crusts of exuda on ears, nose, and tail, it appears that his animals were also sufferin from vitamin B_6 deficiency. In threatened abortion in women he foun the presence of the symptoms to coincide with the development antiproteolytic activity of the serum. By means of large doses wheat germ oil he has reported marked success in the treatment a series of 82 cases of threatened abortion.

Shute's studies are of special interest because he believes he h demonstrated that the antiproteolytic substance in serum in cases threatened abortion is estrin, or estrin-like, since he was able

produce the property of antiproteolytic serum in normal blood adding estrogenic hormones to normal blood. The presence of trin, or some similar substance, in the blood is, he suggests, a direct sult of vitamin E deficiency. He has been able to produce, for a w hours, blood serum having the characteristic antitryptic property injection of a potent gonadotrophic preparation of pregnancy ine. He points out that in premature separation of the placenta ere has never been a satisfactory explanation offered for the failure the placental villi to penetrate the uterine wall as far as the serosal at, since they normally are able to erode their way into the yometrium for a very considerable distance. It has been taken for anted, he says, that there is a certain amount of maternal resistance such encroachment of the fetal trophoblast throughout every regnancy. He suggests that when such resistance is excessive, pregancy ends prematurely. He adduces evidence to indicate that the lacental villi, in both normal and abnormal pregnancy, themselves ossess, and probably secrete, the antagonist to their own erosive gents, i. e., the antiproteolytic substance. It is further suggested that uring pregnancy vitamin E and estrin, or a similar substance, exist some degree in equilibrium and if there is too much of the stin-like substance, pregnancy is interrupted.

Since the number of clinical trials of vitamin E is still small, and ne statistical significance of the data has in no instance been shown, is too early to assert that the place of this vitamin in therapy has een established.

REFERENCES

ATTILL and CONKLIN (1920). J. Biol. Chem., *44*, 137.
VANS and BISHOP (1922). Science, *56*, 650.
VANS (1932). J. Am. Med. Assoc., *99*, 469.
ATTILL (1938). J. Am. Med. Assoc., *110*, 1831.
MMONDS, BECKER and MCCOLLUM (1927). J. Am. Med. Assoc., *88*, 1047.
NES (1927). J. Biol. Chem., *75*, 139.
ATTILL (1927). J. Am. Med. Assoc., *89*, 1505.
ATTILL and CRAWFORD (1930). Indust. and Engineering Chem., *22*, 341.
LCOTT and MATTILL (1931a). J. Biol. Chem., *93*, 65.
RADWAY and MATTILL (1934). J. Am. Chem. Soc., *56*, 2405.
LCOTT and MATTILL (1931b). J. Biol. Chem., *93*, 59.
LCOTT and MATTILL (1934). J. Biol. Chem., *104*, 423.
MMINGS and MATTILL (1931). J. Nutr., *3*, 421.
ADDELL and STEENBOCK (1928). J. Biol. Chem., *80*, 431.
VANS and BURR (1927). Memoirs of the U. of California, *8*.
UMMOND, SINGER and MACWALTER (1935). Biochem. J., *29*, 456.
LCOTT (1935). J. Biol. Chem., *110*, 695.
VANS, EMERSON and EMERSON (1936). J. Biol. Chem., *113*, 319.

Todd, Bergel, Waldmann and Work (1937a). Nature, *140*, 361.
Todd, Bergel, Waldmann and Work (1937b). Biochem. J., *31*, 2247, 2
Emerson (1938a). J. Am. Chem. Soc., *60*, 1741.
Emerson, Emerson and Evans (1936). Science, *83*, 421.
Emerson, Emerson, Mohammad and Evans (1937). J. Biol. Chem., *122*,
Fernholz (1937). J. Am. Chem. Soc., *59*, 1154.
McArthur and Watson (1937). Science, *86*, 35.
Fernholz (1938). J. Am. Chem. Soc., *60*, 700.
Bergel, Jacob, Todd and Work (1938). Nature, *141*, 646.
Karrer, Fritsche, Ringier and Salomon (1938). Helv. Chim. Acta,
520.
Smith, Ungnade and Prichard (1938). Science, *88*, 37.
Emerson (1938b). Science, *88*, 40.
John, Dietzel, Günther and Emte (1938). Naturwissenschaften, *26*, 3
Evans, Emerson and Emerson (1938). Science, *88*, 38.
Evans and Bishop (1922). J. Metabolic Research, *1*, 319, 335.
Mattill and Stone (1923). J. Biol. Chem., *55*, 443.
Sure (1924). J. Biol. Chem., *58*, 693.
Mason (1926). J. Exper. Zool., *45*, 159.
Long and Evans (1922). Memoirs of the U. of California, *6*.
Urner (1931). Anat. Record, *50*, 175.
Mason (1933). Am. J. Anat., *52*, 153.
Card, Mitchell and Hamilton (1930). Proc. Poultry Sci. Assoc., 22
Meeting.
Adamstone (1931). J. Morph. & Physiol., *52*, 47.
Barnum (1935). J. Nutr., *9*, 621.
Evans (1928). J. Nutr., *1*, 23.
Blumberg (1935). J. Biol. Chem., *108*, 227.
Ringsted (1935). Biochem. J., *29*, 788.
Emerson and Evans (1937). J. Nutr., *14*, 169.
Olcott and Mattill (1937). J. Nutr., *14*, 305.
Martin (1937). J. Nutr., *13*, 679.
Evans and Burr (1928). J. Biol. Chem., *76*, 273.
Marchesi (1935). Boll. Soc. ital. Biol. sper., *10*, 984.
Burr, Brown and Moseley (1937). Proc. Soc. Exper. Biol. & Me
36, 780.
Lipshutz (1936). Revue neurol., *65*, 221.
Olcott (1938). J. Nutr., *15*, 221.
Morgulis and Spencer (1936). J. Nutr., *11*, 573.
van Wagenen (1925). Anat. Record, *29*, Suppl., 398.
Nelson (1933). Anat. Record, *56*, 241.
Rowland and Singer (1936). J. Physiol., *86*, 323.
McQueen-Williams (1934). Anat. Record, *58*, Suppl., 77.
Singer (1936). J. Physiol., *87*, 287.
Barrie (1937). Nature, *139*, 286.
Evans (1928). Am. J. Physiol., *85*, 149.
Davidson (1935). Canad. Med. Assoc. J., *32*, 364.
Haddow and Russell (1937). Am. J. Cancer, *29*, 363.

AMSTONE (1936). Am. J. Cancer, *28*, 540.
WNTREE, STEINBERG, DORRANCE and CICCONE (1937). Am. J. Cancer, *31*, 359.
-COLLUM, BECKER and DAY (1938). Unpublished data.
CHARACH and ALLCHORNE (1938). Chem. and Industry, *57*, 600.
CHARACH (1938). Nature, *142*, 35.
LMER (1937). Indust. and Engineering Chem. (Anal. Ed.), *9*, 427.
IOMAS, CANNON, MCNUTT and UNDERBJERG (1938). J. Nutr., *15*, Suppl. 10.
HIOPPA (1936). Ztschr. f. Vitaminforsch., *4*, 167.
Y and VOGT-MØLLER (1934), Vet. J., *90*, 288.
OGT-MØLLER (1931). Hospitalstid., *74*, 567; id. (1933), Acta Obstet. Gynecol. Scand., *13*, 219; id. (1936), Klin. Wchnschr., *15*, 1883.
URRIE (1936). Brit. Med. J., *i*, 752.
URRIE (1937). Brit. Med. J., *ii*, 1218.
ATSON (1936). Canad. Med. Assoc. J., *34*, 134.
IUTE (1935). J. Obstet. Gynecol., *42*, 1071, 1085; ib. (1936), *43*, 74; id. (1937) Am. J. Obstet. and Gynecol., *33*, 429.

CHAPTER

XXIV

Dietary Properties of Foodstuffs

AN OUTSTANDING feature which emerged as nutritional
search developed during the present century was the difference
nutritive properties of individual foodstuffs which, according to
chemical procedures for analysis in vogue at the end of the ninetee
century, appeared to be closely similar in composition. Many fa
have been recorded in preceding chapters which make clear w
foods which yield similar amounts of protein, carbohydrate, and
often differ greatly in food value. Marked differences in the biologi
value of proteins, composition of mineral content, and of vitami
account for these variations in quality of foods from the physiologi
standpoint. In this chapter is presented a brief summary of the dieta
properties of the more important natural and manufactured produ
that enter into the human diet. These data afford a basis for
termining the extent to which generalizations can be safely ma
as to what foods have supplementary values, and indicate the typ
of combinations which tend to make diets complete for the supp
of nutrition.

The Cereal Grains.—The principal cereal grains which are
cluded in human dietaries comprise, in the order of the magnitu
of their production and consumption, rice, wheat, maize, rye, barl
millet, and oats. These grains consist of a germ constituting t
embryo plant, which is rich in protein and oil, and in the case
wheat at least is devoid of starch; an endosperm, consisting principa
of starch but containing some proteins; an aleurone layer surroundi
the endosperm, which is closely packed with protein aggregates; a
a pericarp which forms a skin about the seed. The pericarp, wi
adherent particles of aleurone layer and of endosperm, constitute t
bran that is separated in milling.

That the cereal grains serve as food for man and animals is a nance occurrence, the purpose of the seed being to produce a new lant. The substance of both germ and endosperm is designed for lant, not animal food. Experiments show that the proteins of cereals ave a relatively low biological value, in that they can be utilized for ansformation into animal proteins during growth only to the extent f about 25 per cent. The vitamins seem to be largely concentrated the germ of the cereals. The bran layer is rich in phosphorus, but is relatively unavailable. The endosperm is relatively poor in in-ganic elements except potassium which is fairly prominent. Some each of the physiologically important mineral elements are present, owever, in every part of the grain.

Certain generalizations can be made concerning the dietary operties of these separate parts of the cereal grains. The proteins the coarser material removed in milling, i. e., the bran and mid-ings of wheat, being derived largely from the aleurone layer, prob-ly have a somewhat higher biological value than do those of the archy endosperm which constitutes the highly refined flour. The oteins of the germ are also probably better constituted as respects imal nutrition than are those of the endosperm. We have but little finite knowledge on these points, however, so only general tements are warranted.

Yellow coloring matter in a cereal indicates the presence of some otenoid, usually carotene, which is a source of vitamin A. In llow maize, however, the principal yellow pigment is cryptoxan-ne, which also is a source of vitamin A. Yellow color also goes ng with the distribution of riboflavin. Since this substance has en known less than four years, and methods for its quantitative termination in many kinds of food materials have not been fully veloped, we lack any reliable data as to how much of it is present grains. We do know that it is present in all the cereals named ve. White varieties of maize and the white endosperms of rice d oats are essentially lacking in carotene and other vitamin A cursors but it is possible that they contain at least a small quota riboflavin. Both these pigments are much more abundant in the ms of all cereal grains. Likewise, thiamin and nicotinic acid, and other vitamins of the B-complex, are much more abundant in the m than in any other part of cereal grains. The practice of discard-the germ in milling operations employed in preparing cereal ducts for human consumption is unsound from the dietary ndpoint.

Wheat.—From the dawn of history wheat has been the dominant eal food of the white race. Our earliest ancestors probably pounded wheat grains in mortars to make cooking easier, and at some

remote period, crude mills were invented which employed principle of grinding the grain between two stones, the upper revolving upon the lower. These were grooved radially to carry particles from the center outward, and during thousands of ye almost no advance was made in separating the different parts of seeds. Stone grinding tended to pulverize all parts of the grain, the bran layer ground with difficulty, so that it was always poss to sift out some of the coarser particles and thus prepare a fl which had a higher digestibility than had the whole grain prod In the Old Testament "fine" flour is distinguished from the en wheat flour. It was not until 1879 that the modern roller mill proc was invented for milling wheat. In principle this is accomplish by passing the grain between steel rollers which crush the grain pressure instead of grinding it. The first rollers are corrugated a do not touch, and one revolves faster than the other. The grain dried to make it brittle; and before milling it is exposed to moist to enable the outer layer only to absorb moisture. Hence the perica becomes tough and flexible and is removed in the form of flak which are easily separated mechanically. The crushed grain is la passed between smooth rollers which complete the pulverizi process, in which the germs, being rich in oil and protein, a plastic, are also easily separated because of their size and shape. T roller process makes possible, when combined with repeated siftin through screens and bolting cloth, the manufacture of a flour whi consists entirely of starchy endosperm with its finely crushed protei White flour represents starch, endosperm proteins, gliadin a glutenin, together with some ash substance, principally potassiu White flour is a good source of energy in the form of starch a protein. It is essentially lacking in all the vitamins but in the u bleached state probably contains a little carotene and riboflavin. T widespread practice of bleaching white flour destroys these pigmer and makes a vitamin-free flour. Our liking for bread made fro white flour is an example of the failure of the appetite to guide correctly, since coarser flours have higher nutritive value. An i portant incentive for the manufacture of white flour is the improv ment of its keeping properties under modern conditions of fo distribution.

Insects which infest grains tend to deposit their eggs in the ger which is the best part of the seed from the nutritional standpoi Hence, whole wheat flour, which contains the germ, tends to harb weevils when it is stored through warm weather. White flour w not support the life of insect larvae. After wheat is milled the oxyg of the air has access to the oil in which the germ is rich; this oil ten to become rancid, spoiling the flavor of the flour. Whole wheat flo

annot be handled in commerce without danger of deterioration since he wheat-growing regions are generally far from the centers of opulation. The keeping properties of highly milled flour are a great dvantage to the distributor and the baker. Whole, unmilled wheat, an be shipped long distances more safely than can flour for the easons cited. There are sound reasons why the population should ontinue to use white flour as a breadstuff; these rest on commercial xpediency.

Our liking for the familiar white wheat flour loaf depends on its aste, which is in great measure due to the growth of yeast in the eavening process and the development of aroma during baking ogether with its peculiarly attractive chewing properties. Wheat our proteins have the remarkable property of forming, when wetted nd kneaded, a dough, which when leavened with a yeast culture ntraps the carbon dioxide produced by yeast fermentation of carbo- ydrate and results in a dough of spongy texture, and hence a spongy oaf after baking.

The tendency in recent years has been to incorporate skim milk nstead of water into flour to make dough. This practice is laudable nce it makes for a better tasting and more nutritious bread. White read has been much maligned as a food by unthinking critics. he proliferation of yeast in leavening and the introduction of skim nilk both add to the value of bread, and even though we admit nat it is still an incomplete food, it is a wholesome component of he diet. When menus are properly planned, bread may be freely aten without in the least detracting from the quality of the dietary. should be remembered that nearly all other foods, considered ngly, are deficient in certain indispensable nutrients, and that we an secure a highly satisfactory diet only by combining a number f kinds of foods in such proportions that they make good each other's eficiencies and form a "balanced" dietary.

While whole wheat bread is more nearly complete nutritionally han is a bread made from white flour, this is a matter of little ractical importance under ordinary conditions where a mixed diet employed. Whole wheat bread has a distinctive flavor which is referred by some but not by most people. It, too, is a wholesome ood when eaten in moderate amounts in proportion to the entire ood supply, but it is by no means satisfactory when eaten as the rincipal food. This was shown by the experience of certain European eoples during the World War when food shortage necessitated xcessive restriction of the diet to bread which was made from nearly hole wheat flour. The nutritive value of the diet largely composed f bread was low and the coarseness of the bread caused intestinal isturbances which were widely complained of.

Bran.—Bran has laxative properties; it increases the bulk of digestible residue in the intestine and tends to correct certain conditions of faulty bowel elimination. It is questionable whether bran is as desirable a source of indigestible fibre as are leafy vegetables such as cabbage, spinach, lettuce, and numerous others. These, when properly cooked, afford water-holding indigestible residues which are less harsh and less likely to irritate the delicate lining of the digestive tract than is bran.

Wheat Germ.—Wheat germ contains better proteins than does the endosperm of wheat, and its oil is the richest known natural source of vitamin E. The germ is likewise rich in vitamins of the B-complex, being nearly equal to yeast in this respect. Wheat germ is rather difficult to keep long without its becoming rancid, but when much of the oil is pressed out and the germ is packed in vacuum tins it serves as an excellent vitamin-rich supplement to the human diet and might well be more extensively eaten as an adjuvant to many diets.

Rice.—Rice contains less protein than does wheat, but is otherwise very similar to wheat in its dietary properties. Rice is the cereal of the swamp and is produced abundantly in regions where wheat cannot be grown. It is the principal cereal of far more people throughout the world than is wheat. Rice is eaten mostly in the polished state. Polishing is accomplished by abrasion, which removes the pericarp and aleurone layers as well as the germ, leaving the endosperm intact in the form of the familiar white rice of commerce. Polished rice is similar to refined wheat flour in its dietary properties. It is a source of energy and protein but lacks all the vitamins and contains little of any inorganic element with the exception of some potassium.

Rice is polished for the same reason that wheat is converted into refined flour: viz., because it is more appealing to the taste, and polished rice keeps better and does not develop stale flavor. A considerable amount of rice is prepared for consumption by steaming the red rice to gelatinize the starch near the surface of the kernel and subsequent drying in the sun before polishing. Steamed red rice is eaten in a condition which retains some of the outer layers and germ, and thus provides some of each of the vitamins of the B-complex. It is, therefore, superior to polished rice. In addition some rice is consumed as "under-milled" rice; this also contains some adherent pericarp, aleurone layer, and germ, and contains sufficient of the B-complex to prevent beriberi.

Rice, therefore, requires supplementing with such foods as will make good its deficiencies. These supplements are of the same nature as are those required for wheat flour, viz., foods which provide protein

undant in the essential amino acids, foods rich in the essential ineral elements and in the vitamins.

Maize.—Maize culture was restricted to the Americas until the iscovery by Columbus, after which it was introduced into Italy, umania, Egypt, and other areas where climatic conditions are vorable to its growth. A summer rainfall which is favorable to the evelopment of a maximum yield of maize is ruinous to a wheat op. The climatic conditions favorable to maize growing are found nly in certain areas throughout the world, and these are not the eas where wheat or rice can succeed as farm crops. Climate and il conditions determine to a remarkable degree the kinds of agri-ltural products which can be grown, and in earlier times determined e character of the diet of peoples to a much greater extent than nder modern conditions of transportation.

The North American Indians prepared hominy from maize by eating the grain with a water extract of wood ashes containing otassium carbonate and hydroxide sufficiently alkaline to soften the ard pericarp. After washing away the alkali the grains were boiled n water, which made them soft and appetizing. Early milling prac-ces for maize involved grinding the entire kernel between millstones, ut methods have been refined so as to produce cornmeal free from erm and pericarp.

The dietary properties of refined cornmeal are so similar to those f white flour and polished rice that they need not be enumerated. s respects maize there are greater differences than in rice or wheat our which are dependent upon color. There are white, yellow, red, nd blue varieties of maize but cultivation is in great measure re-ricted to white and yellow kinds. Yellow maize makes yellow efined meal. There is in the maize kernel a certain amount of boflavin. White maize is deficient in cryptoxanthine. The potential itamin A value differs so greatly between yellow and white maize at the feeding value of the former is distinctly superior to the latter, fact which was observed by swine producers many years before utritional investigations led to the discovery of vitamin A, and even arned animal husbandrymen long questioned the testimony of ractical feeders on this point.

As has been pointed out in the discussion of pellagra, it seems to e demonstrated that a deficiency of nicotinic acid plays an important, not the determining, role in the prevention of this disease, which as been confined almost entirely to populations subsisting largely pon maize meal. This fact indicates that maize is deficient in icotinic acid as compared with other grains. It seems remarkable at excessive eating of polished rice and of white wheat flour does ot lead to pellagra. The experience available does not offer an

explanation of this problem, which may possibly be that nicot acid is present to some extent throughout the endosperms of b wheat and rice; or it may be that wheat and rice eaters alm invariably eat sufficient amounts of other foods containing the pella preventive factor, whereas maize eaters are less likely to do so. subject invites further study.

Barley and Rye.—These grains serve as bread cereals in m parts of the world. Rye produces about twice the yield per acre does wheat, and rye and barley varieties yield well under clima conditions where wheat does not. The dietary properties of rye a barley are very similar to those of wheat, but they produce bre of very different texture from wheat because their proteins poss inferior dough-forming property. These grains are always less hig milled than is wheat and yield reddish or brownish flours wh form weak dough and can be leavened only to a small extent, co sequently the resulting bread is hard. Most of the rye now go into the manufacture of beer. Barley is the principal bread gra in semi-arid regions, but in America it is now principally used soups and in infant foods.

Oats.—Coarse-ground oatmeal was first used as a principal cer food for man in Scotland, but machinery has been perfected f making rolled oats which is now used extensively as a breakfast foo Most of the oat crop of America is fed to animals, only about 4 p cent being used as human food. Dr. Johnson's famous definitio for oats: "A grain fed to horses in England, but eaten by men Scotland," on which a Scot is said to have commented: "And pra where do we see such men as in Scotland and such horses as England?" suggests that the oat is a superior food cereal. This vie has not been supported by experimental results.

Rolled oats are deficient in certain of the heat-stable vitamins the B-complex. Oats are as good a source of thiamin as are oth whole cereals, but judging from their appearance are free or near free from riboflavin. We know nothing of the content of the o kernel in nicotinic acid, or vitamin B_6, but it appears that from th nutritional standpoint for the rat one of these three vitamins form the limiting factor in this grain.

The biological value of oat proteins closely parallels that of th other cereals, but its content of fat is somewhat higher. Like a other cereals, in terms of the nutritional requirement of the rat it deficient in calcium.

Millet, Sorghum, and Buckwheat.—These grains serve as huma food in certain regions, but only to a small extent as compared wit wheat, rice, maize, rye, barley, and oats. Millet is eaten in Nort China, parts of Russia and other regions. It is an inferior grain

espects its physical properties. The seed coat is extremely hard. fillet is eaten as human food largely where only highly drought-esistant grains can be grown.

Sorghum is another drought-resistant plant of the grass family, nd is principally used for feeding animals, especially poultry, because f its unappetizing character for humans. It is eaten by man in some arts of South Africa. Its nutritional properties appear to be like ose of other cereals.

Buckwheat is grown on poorly drained and acid soils where her plants of economic value do not thrive, and is widely cultivated Northern Europe. It is popular in America as a pancake flour hen mixed with wheat flour. Few studies have included buckwheat, ence its dietary properties are not exactly known. It has one pecu-arity which should be noted, viz., that of containing a fluorescent e, which, when eaten, sensitizes persons with fair skins to light, using what is vulgarly called "buckwheat itch." If albino animals guinea pigs, rabbits) are fed liberally on buckwheat and are then posed to sunlight, they quickly die. Pigmented animals are not riously affected. There are several reports in the literature of cattle ing injured by feeding on buckwheat as a forage plant when they and out in the sunlight. When green buckwheat hay is fed to imals kept in the shade no injury results.

Legumes—Peas and Beans.—Peas and beans, including the soy an, are consumed in enormous amounts by many peoples. They ve high protein contents and, with the exception of the soy bean, low content of fat. Their proteins are without exception of low ological value, but it is not known which amino acids are lacking these proteins. Pea and bean proteins, including soy bean, do not ake good the amino acid deficiencies of the cereal proteins and cordingly are not good supplemental foods in this or other respects. Qualitatively the vitamin content of these seeds appears to be mplete, but it is not high, being apparently of the order of the hole cereals. The mineral content resembles that of seeds in general, ing low in calcium but containing some of each of the essential organic elements. Like other plant products their mineral content ries greatly with the nature of the soil on which they are grown. Most, if not all the beans, contain large amounts of certain hemi-lluloses which undergo fermentation easily in the alimentary tract th gas formation, principally methane and carbon dioxide. If eaten ely they tend to induce flatulence and for this reason should not eaten in excess. Rats fed largely on navy beans become greatly stended from ballooning of the stomach and intestines.

Soy beans are used principally by rice-eating peoples, and serve the principal protein supply when simple dietaries are taken.

There seems to be little supplementary value in soy bean proteins those of rice; accordingly, their value is independent of any impro ment in utilization of rice proteins. Soy beans are rich in an oil a therefore of high caloric value.

Tuber and Root Vegetables.—In this class of vegetables included the potato, sweet potato, taro, manioc, turnip, radish, car beet, parsnip, and many others. They have in common the prope of being fleshy roots or stems specialized as storage organs for star to which fact they owe their energy value. All are markedly defici in protein, and so far as is known such protein as they contain little supplementary value in improving the utilization of cer proteins, in the sense of supplying essential amino acids in wh cereal proteins are deficient.

Distinction in dietary properties is made in these foods on basis of color. Yellow varieties contain carotene; accordingly th serve as sources of vitamin A, which is not the case with wh varieties. We know nothing as yet concerning the distribution tuber and root vegetables of riboflavin, nicotinic acid, and vitam B_6, but it is known that they all contain some thiamin. Likewi when in the uncooked state, they invariably contain considera ascorbic acid, hence they are antiscorbutic foods. This substance largely lost on cooking, the extent depending on the degree to whi oxygen of the air has access during the process. The red color beets, apparently, is without nutritive significance.

Fruits.—The more highly prized fruits have flavors which ma them especially attractive to the palate. Otherwise, fruits closely semble the tuber and root vegetables in their dietary properties. Th antiscorbutic value is outstanding, when eaten uncooked. The citro fruits are especially rich in ascorbic acid, apples, pears, peach apricots, etc., having a lower value in this respect. Grapes are of ve low antiscorbutic value. Fruits, as a class, contain more sugar a less starch in proportion to the total carbohydrate than do the tub and roots. As has been pointed out in the discussion of blood regene tion as affected by diet, apricots possess some still unidentifi constituent which makes this fruit unique among the fru studied.

Berries.—Berries of all kinds appear to be closely similar in th dietary properties to the fruits, and no exceptions worth mentioni are known. Berries differ from the fruits, with the exception of fi in containing large numbers of small stony seeds which tend irritate the intestines and cause stimulation of peristalsis. Such see have occasionally been accused of causing inflammation in the a pendix and in the large mucous glands inside the rectum, but appears that they have not frequently been the cause of such inju

Nuts.—Many people with faddist tendencies as respects foods be-
eve that nuts possess unique nutritive value, and that they are
mplete in all the essential nutrients. This view finds no support
experimental studies. All of the commonly used nuts, with the
ception of the chestnut, which is starch-rich and fat-poor, are
tremely rich in proteins and fats, hence they have high caloric
lue and are good sources of protein. In no instance, however, have
eir proteins been found to be of high biological value, this being
the general order of the cereal grains. The quantitative studies
ailable are relatively few, consequently no very exact statements
e warranted about any of them. Their distinctive and appetizing
vors recommend them as human foods, but their high energy
lues point to their consumption in moderation by sedentary persons
ho have a tendency to put on weight. We have at present no data
on which to distinguish in nutritive properties between any of the
ts such as peanuts, walnuts, hickory nuts, hazel nuts, Brazil nuts,
monds, etc. Their vitamin contents are in general practically un-
own. Their mineral composition resembles that of cereals, peas,
d beans.

The Leafy Vegetables.—One of the most important of the earlier
scoveries relating to remarkable differences in the dietary properties
different kinds of vegetable products was that the leaf of the plant
a complete food, whereas none of the storage organs of plants,
eds, tubers, roots or fruits enjoy this distinction. This fact was first
ted by McCollum, who pointed out that this difference was associ-
ed with the difference in function of the leaf as contrasted with the
ecialized structures serving as storage organs containing reserve food
the young plant of the next generation. The leaf is the site of
nthesis of proteins, carbohydrates, and fats and is rich in actively
nctioning cells. These cells contain everything which is necessary
the metabolic processes, and they supply all the nutrients which
animal requires.

Familiar examples of the adequacy of the leaves of plants for the
trition of animals are the subsistence with unimpaired vigor from
neration to generation of grazing animals such as the bison of the
ains of North America and of the numerous browsing animals
hich eat the leaves of trees, weeds, and shrubs. Cattle and horses
efer grass to any other leafy food, whereas sheep, goats, moose,
affe and many others prefer the strongly flavored leaves men-
ned. Such animals eat enormous amounts of tannins, glucosides,
d other principles which taste bad to grazing animals. Most of
grasses such as make good pastures contain little tannin. The
ld grass which covered the plains of the West before the advent
the plough had a pleasant taste to the human palate. The same

is true of the juice of wheat, corn, oat, and other cereal prod plants now cultivated. Pliny mentions the custom of the A Egyptians of chewing the stems of the papyrus rush, swallowing the juice. In regions where sugar cane is grown it is a co practice of people to chew and swallow the juice. In Mexic juice of the agave is fermented to make pulque. Other examp the use of plant juices as food could be mentioned. Before the prairie grass was destroyed in the Middle West it was a custo farmers in raising young calves to supplement the limited supp skim milk with "hay tea," which was made by steeping well hay in water, the extract being added to the milk. This grass inf was an excellent feed for calves, apparently almost equalling at least, in supplying essential nutrients, especially vitamins mineral salts in abundance.

It is of interest to note that in 1937, through the courtesy of J. S. Hughes, the authors had the opportunity to examine a sa of a leaf flour made from young oat leaves which were harv before stems had developed, dried artificially and ground to a fine, bright green powder which had a pleasant taste. It would that there may be a future in human nutrition for such leaf f which might be made from any of several of the young c plants. Certainly such leaf flours have very high nutritive valu respects all the essentials of an adequate diet.

Kelp and Irish Moss.—Kelp and other marine plants co much more iodine than do land plants or any other edible prod There is also considerable bromine in kelp. Kelp meal has been moted as a supplement for farm rations. Kelp meal used to re a part of a good dairy ration has no beneficial effect on milk duction, health, or reproduction of dairy cows consuming a dairy ration. Dehydrated kelp has no effect on the hemogl content of the blood of chickens, or on the composition of the b as shown by ash determinations of chick bones. The feeding of increased markedly the iodine content of both yolk and whit hen's eggs. It has not been shown that kelp possesses any valu nutritive properties other than that referable to its iodine conten

Irish moss is frequently eaten by people living on the Atl coast. When treated with vinegar it becomes gelatinous and is app ing. There is no evidence that it is of much value as a food ex as a source of iodine. All the evidence available indicates that organic iodine, as found in iodized salt, is as satisfactory for nutr purposes as is iodine in vegetable products.

The Vegetarian Diet.—In all periods in the past of which have any knowledge there have been people who subsisted upo strict vegetarian diet, generally for esthetic and moral reasons.

merous species of animals which are strictly vegetable feeders afford
ly presumptive evidence that such diets are satisfactory for humans.
ttle, horses, and many other animals have remained in health and
gor through countless generations when feeding on grass alone,
fore they were domesticated and were generally given supplements
seed products. The elephant, ox, horse, and buffalo have great
rength and endurance, and thrive on vegetable food alone. These
d other vegetable feeding animals are leaf eaters. The leaves of all
ants appear to be complete from the nutritive standpoint. It is
ite otherwise with seed products, tubers, roots, and fruits. Although
ey contain, apparently without exception, everything that is essential
r normal nutrition, they are too imbalanced in their composition to
ford a satisfactory diet even when eaten in considerable variety.
hus they are all very deficient in calcium, and in goiterous regions
iodine. They supply less than the optimal amount of protein on
count of its relatively low biological value or deficiency of content.
ll white varieties are deficient or lacking in potential vitamin A
lue. These deficiencies may be corrected by the inclusion of protein-
ch nuts, beans or peas, but since the proteins from these sources
e of low value, and do not enhance the proteins of other vegetable
ods, it is necessary to eat an abnormally high protein diet in order
supply enough of the essential amino acids. This results in burden-
g the body with the degradation of excessive protein which cannot
used.

Since the edible leaves are not only complete, nutritionally, in
emselves, but also have supplemental values not possessed by other
oducts of vegetable origin, it is possible, by making suitable combina-
ns in which, irrespective of the other foods used, a liberal quota of
fy vegetables is included. It may be questioned whether any diet
mposed of cereal grains, tubers, roots, fruits, nuts, beans, peas, and
fficient leafy foods, is not more bulky than the human digestive
ct can handle to best advantage. Nevertheless, modern nutritional
searches make clear why subsistence by man on a strictly vegetarian
t is possible, but it cannot be asserted on any evidence known
the authors that such a diet will prove as satisfactory as does a
xed one.

The lacto-vegetarian diet, or combination of vegetable foods and
lk, is, however, easy to plan so as to be highly nutritious, and to
omote optimal health. The same can be said of the ovo-vegetarian
t provided the egg moiety is sufficient. We cannot assert with
nfidence at present just what proportion of milk or eggs in an
erwise vegetarian diet constitutes the minimum for safety or the
imum for quality. Unless a liberal quota of leaf food is included
ovo-vegetarian diet will be poor in calcium. A safe assumption

would seem to be that 25 to 35 per cent of the total calories shou come from the ingredient of animal origin. If it were practicab glandular organs, such as liver, kidney, sweetbreads, could be used the sole supplements for a vegetable diet, but these, like eggs, conta too high a proportion of phosphorus to calcium and too little absolu amounts of calcium for best results. Muscle meats are less valuab supplements for vegetable foods than are milk, eggs, and glandu organs, since they are less rich in most of the vitamins, contain poorly constituted mineral mixture that is low in calcium.

Foods of Animal Origin.—*Milk:* Milk and eggs are the on foods which are designed by nature for the nourishment of t young. Experiment shows that they are excellently constituted f this purpose and are equally valuable for adults.

The milk of cows, goats, camels, mares, or sheep has been an is the most prominent article of diet of people living in dry regio where pasturage is the sole or principal agricultural resource. Th mode of living is discussed in the chapter on the dietary habits of ma

It is a remarkable fact that the proteins of milk, eggs, glandul organs, and muscle meats are all so constituted as respects the amino acid content that certain of these "building blocks" are preser in much higher proportion than they occur in any vegetable foods wit the possible exception of leaf proteins. Vegetable proteins, therefor cannot be efficiently transformed into animal proteins, much of th former being required to make less of the latter. It is on this basis tha the value of animal proteins as supplemental proteins rest. They pro vide in relative abundance certain amino acids which are deficient i proportion to the body's needs in vegetable proteins generally. Mil proteins are of high value in enhancing the value of vegetable pro teins.

Milk is the only food of animal origin which is rich in calcium Since this mineral is generally provided in inadequate amounts b vegetable foods other than leaves, milk occupies a unique position a a source of this element.

Milk contains a full quota of the vitamins, but when pasteurized or otherwise heated it loses ascorbic acid. Its content of vitamin D is far below the nutritive requirements of the young and probably also of the adult. In sunny regions this is not a disadvantage, but i temperate and colder regions a supplement of vitamin D is necessary

Milk is deficient in iron, copper, and manganese, but apparently provides all other essential inorganic elements in sufficient amounts Pasteurized milk picks up some copper in the process and is sufficien as a source of this element. These elements are, however, generally provided in sufficient amounts by vegetable foods when a judicious selection of these is made.

Eggs: The egg contains much protein and fat and only about 1 r cent of carbohydrate. It is, therefore, unbalanced from the nutritive ndpoint, but it is a complete food qualitatively, especially the yolk. xperimental animals have been fairly successfully raised on egg lk as the sole source of nutrient. Egg alone does not constitute a tisfactory diet. As a supplemental food it is probably the equivalent milk except in respect to calcium and ascorbic acid, in which it is ficient. The developing chick absorbs much of its calcium from the ell, which is discarded when we eat eggs. Eggs contain much more tamin D than does any other ordinary human food. The amount pends greatly on the amount of this substance in the feed of the hen hich transfers to her eggs either the natural form of vitamin D as it curs in fish liver oils or calciferol when these are provided in undance in her feed.

Eggs are rich in cholesterol in which milk is relatively poor. Since eral feeding of cholesterol to rabbits and perhaps other herbivorous imals produces changes in the arteries characteristic of arteriosclesis, the opinion has been voiced from time to time that free eating eggs may be detrimental to the arteries. This view rests on very nder evidence and it seems highly improbable that the amount of olesterol ingested when one eats an egg or two a day would do y harm.

Muscle Meats.—Since the edible portion of the carcass consists gely of muscles this form of meat predominates in the omnivorous d carnivorous types of diet. A diet of muscle meats only is wholly adequate for maintaining adequate nutrition. This is due to its ficiency of calcium, its disproportionately high content of phosorus, its deficiency of thiamin, and perhaps other vitamins of the complex, and of all the fat-soluble vitamins. Muscle meats are good urces of protein of relatively high biological value and meat proteins e good protein supplements for vegetable proteins. Their flavor o recommends them highly.

The Glandular Organs.—The liver, kidney, pancreas, thymus, d other glandular organs, with the exception of the thyroid, have ceptionally high nutritive values. They are complete nutritionally, cept for deficiency of calcium. Their content of the vitamins, with e exception of vitamin D, is higher than in any other foods except rhaps the leafy vegetables. Liver and kidney, in particular, contain uch of the intrinsic and extrinsic factors which are necessary for od formation, hence these are largely relied upon (especially liver) sources of the concentrates which the pernicious anemia patient ust take throughout life.

CHAPTER

XXV

Appetite, Normal and Perverted

DISTINCTIONS Between Appetite and Hunger.—Appetite an hunger are distinct physiological manifestations. Hunger is cha acterized by a peculiar dull ache, referred to the epigastrium, whic may pass into a highly uncomfortable pang or gnawing sensatio associated with drowsiness, lassitude, faintness, or headache. Canno of Harvard and Carlson of Chicago have studied extensively th physiology of the empty stomach. The subject is thoroughly discusse by Cannon ('34), Carlson ('19), and Morgulis ('23). It has bee shown that in hunger there are painful contractions of the gastri muscles, which may fuse into a continuous cramp. With each perio of activity, which may comprise widespread contractions of the diges tive canal, there may be a pouring out of bile and of pancreatic an intestinal juices. But generally gastric juice is not secreted at thes times. Hunger is usually absent in fever and in infection, with sys temic involvement, because of a reduction in movements of th alimentary canal. Boldireff (cited by Cannon) found that when dog were fatigued, the rhythmic contractions failed to appear. The experi ence of many people of being "too tired to eat," is thereby given rational explanation.

Cannon states, "The view has been propounded that appetite i the first degree of hunger, the mild and pleasant stage, agreeable i character; and that hunger itself is a more advanced condition, dis agreeable and even painful—the unpleasant result of not satisfying the appetite." He points out, however, that appetite is related t previous sensations of taste and smell of food, and that pleasant o unpleasant tastes and odors, associated with certain edible substances determine the appetite. There may be pleasure in both anticipatio and realization of eating savory foods. Therefore, psychic element

involved and perversions of appetite may occur, with consequent effects.

That one may experience hunger without appetite is possible. Sternberg has pointed out (cited by Cannon), "hunger may be ficiently insistent to force the taking of food which is so distasteful t it not only fails to rouse appetite, but may even produce nausea." hen, for example, a famished soldier can secure no food except e meat and half-spoiled bread, hunger, but not appetite, impels n to eat the distasteful nourishment. As Carlson (l.c.) has stated, only vague or mild hunger is present, the appetite elements upy the high seat in consciousness; when hunger becomes markedly nful, attention is focused on this element."

Appetite may still persist when hunger is satisfied. We eat with asure the tempting dessert, even when we have already satisfied nger and do not need more food. The age-old custom of serving succession a series of appetizing courses makes it difficult for the lthy person to limit his eating to the point of comfortable repletion, e the appetite still remains, and invites eating to satiety. The ctice of the Roman banqueters in Nero's time of absenting themes from table to empty their stomachs through emesis, only to rn and again enjoy the delights of the palate, is a case in point. That the appetite of the normal individual calls for eating beyond immediate needs of the body is a matter of common knowledge has a survival value under primitive conditions when food is not ays available. The body is provided with a mechanism for storing bohydrate beyond immediate needs in the form of glycogen in liver and muscles; the energy thus stored is available for slow ization when the next meal is delayed, or for survival, as the lt of excessive exertion in running or fighting in case threatening ger is encountered. A more concentrated form of fuel food is which is deposited in depots in the well nourished as a safeguard inst an unlucky day when primitive man might not be able to re food. Yet in our day, the urban dweller, serene in his confidence in the protection of governmental agencies and hence not haced by a need for flight or combat, still retains his primitive etite which, in the weak-willed, often leads him to eat as if paring for a flood. The girl whose admirers keep her supplied n confections, although lolling in comfort throughout a summer ation, is tempted by her appetite to eat more or less continuously weet foods, irrespective of her bodily needs. The idle are notoriy addicted to eating as a pastime and scarcely ever experience ly strong hunger sensations.

Extremes in Dietary Practices.—Faddists, on the other hand, elop singularities of appetite. To the ardent vegetarian the flavor

of a juicy beefsteak may be nauseous, while he shows grim satisfac in masticating his herbs. Such people often exhibit pleasure in den themselves the more appetizing foods. This savors of acquiring m through self-denial. Curious views are met with as to the alle special healthfulness (wholesomeness) of particular foods, such nuts, or fruits, in persons who, in their zeal to attain perfection health, tend to restrict their food supply to a few articles. T appetites are perverted through complex psychic processes. In light of modern knowledge of the necessity for health of a long of specific food factors, it is easy to understand that such fadd may come to have actual perversion of appetite. Their case may comparable to that of the proverbial sternness of the early New E land Puritan subsisting perforce upon "the bean and the cod," probably in a state of chronic malnutrition from several deficienc The situation of the Puritans was parallel to that of the Athen public, referred to in the "Knights" of Aristophanes as "bean-fed su Demos."

Throughout history, appetite has been an antagonist to be fou against and conquered. The debilitated banqueter has often been preacher of the virtues of abstemiousness, as others who have so "wild oats" have been the champions of moderation and grace.

King David and King Solomon lived merry, merry lives,
They had many, many concubines and many, many wives,
But when they both grew older they had many, many qualms
King Solomon wrote the Proverbs, and King David wrote the Psalm

A modern example of the champion fighting the devil of appet at close range was Gladstone, who may be taken to typify the midd aged dyspeptic in failing health. He gave a powerful impulse mastication in England by attributing his own success in life larg to the fact that he had always made it a rule to give every tooth chance, and had applied 32 bites to every morsel. Astute statesm that he was, he failed to discern that, with a full dental compleme 16 chews would have served his purpose.

Dietetic theories have often been propounded by those wh metabolism is imperfect. Like Gladstone, Horace Fletcher preach salvation through control of the appetite by excessive masticati He summed up his philosophy in the meaty statement: "If you only when you have earned an appetite, masticate your food th oughly, and take great care to eat only what your appetite approv the rest will take care of itself." Mr. Fletcher made of masticati an art and a new theory and discipline of life. The number of disciples and their enthusiasm for the new system of eating g

pause who would sweepingly condemn it. The principal claims Fletcherism, as deliberate and prolonged mastication came to alled, were: increased pleasure derived from eating starchy foods, e they become appreciably sweeter the longer the process of ary conversion of starch into maltose proceeds; satisfaction of appetite with a smaller consumption of food; decreased desire protein because its taste was said not to be improved by prolonged ving; better utilization of food when thoroughly masticated, be- e of better digestion and absorption. It is not our concern here onsider the soundness of the statements made by adherents to cherism, but only to call attention to this method of satisfying appetite without overburdening the digestive and excretory sys- s with an overplus of food. At a well-known Middle West torium a chewing song was composed, and the patients were said ave taken innocent delight in singing it at their popular munching ies.

Appetite as a Guide to the Selection of Food.—In Chapter VII have mentioned that phosphorus deficiency in cattle may cause n to develop the habit of bone-chewing, and that the provision of element in any available form quickly causes the disappearance he abnormal behavior. Eccles and Palmer state that cows made cient in calcium will not chew bones in order to get this element. ling ('32), experimenting with parathyroidectomized rats, in ch blood calcium is unstable and tends to fall rapidly when a high of phosphorus is absorbed, states that the animals refused to diets which were rich in phosphorus, but ate freely of the same when its content of phosphorus was reduced to a low level. se observations indicate that, as respects certain dietary factors, appetite calls for what is lacking, and also that a feeling of ill-being, umably experienced by Shelling's rats, caused by taking a food or which produces metabolic disturbances, promptly reflects itself efusal of the offending substance.

Evvard ('29) devised a system of feeding swine which was known he free-choice, or "cafeteria" system. It involved the provision of ral feeding-stuffs having very different composition and nutritive ities (e. g., shelled corn, tankage, linseed oil meal, and chopped fa) in separate containers, so the swine could eat of each as the tite directed. Evvard quotes highly favorable comments on this m from many experiment stations throughout the world. How- the method has been generally discontinued in animal husbandry e the cost of raising marketable swine is higher than in the m of feeding mixed rations designed to produce a maximum of with a minimum of cost for feed and labor.

Osborne and Mendel ('15), in their studies of the nutritive value

of proteins in growth, observed that a diet adequate in other res but containing only 9 per cent of casein, did not support gro But when the ration was supplemented with a small addition of amino acid cystine, growth proceeded at once. They noted that the rats eaten more of the unsupplemented diet, they would secured the requisite amount of cystine and could have grown that been the only required dietary correction. A rat receiving a containing 9 per cent casein plus cystine equivalent to 3 per cen the casein, grew 400 per cent more than did the same animal on same diet without cystine in a preceding period, although it consu only 8 per cent more food in the later period. Other rats w observed to grow well on low protein diets when consuming more calories than those which were not growing because of deficie of an amino acid. These investigators stated that apparently a mar deficiency in any essential ingredient of diet does not lead to a responding compensatory increase in food intake: "They appare limit their feeding to the amount of food yielding approximately requisite energy."

In further studies Osborne and Mendel ('18) observed that wh rats were given the choice of two diets, one inferior and one super the animals had considerable success in choosing the better mixtu

Experiments with Mineral Consumption as Affected Appetite.—Richter ('36) found that the average length of life of adrenalectomized rats was 11 days on a diet probably containi somewhat less than a normal amount of sodium chloride. A gro of 26 adrenalectomized rats fed the same stock diet supplement with 1 per cent of sodium chloride had an average survival peri of 17 days. Thirteen similarly operated animals on the latter sto diet, which were offered a choice between tap water and a 1 cent sodium chloride solution, showed a survival period 69 per c greater because of their choice of drinking water containing the s Five rats after operation were kept on the diet unsupplemented w sodium chloride and were given a choice between tap water and per cent sodium chloride solution. They drank 6 times as much the salt solution after adrenalectomy than before, and their surviv time was increased by 80 per cent. Although no data are given the reliability of the surgical procedure it is apparent that af adrenalectomy the appetite for sodium chloride is greatly increas and that in this circumstance, in rats, it is a guide to the physiologi needs.

Richter and Eckert ('37) have sought to learn whether, as in t case of adrenalectomized rats, appetite is generally a guide to nutriti needs created by glandular deficiencies. Inasmuch as an increase the calcium need of parathyroidectomized rats is well established,

study was made of the level of calcium appetite in these animals before and after removal of the parathyroid glands. Eighteen rats were used in the experiment. They were kept in individual cages containing a food cup and two sources of water, one containing tap water, the other a 2.4 per cent calcium lactate solution. The animals were given a standard adequate dry diet containing 2.56 gm. of calcium per kilogram of food. During 10 days preceding operation the daily intake of tap water per rat was 19.5 cc., as against 14.7 cc. in the 10-day period 30-40 days after operation. The average calcium lactate solution taken during the 10-day period before operation was 1.4 cc., as against 18.9 cc. daily in the period 30-40 days after operation. During the 10-day period 30-40 days after operation the average consumption of calcium was 3.9 times as great as in the preoperative 10-day period.

When 6 parathyroidectomized rats were treated with parathyroid implants, there was a decrease in the intake of calcium lactate solution, approximately to the original preoperative level. In other experiments parathyroidectomized rats were offered a choice between a calcium lactate and a sodium phosphate solution. It was found that the increase in appetite for the calcium solution was accompanied by a definite decrease in sodium phosphate intake. These results were explained on the assumption that the sense of taste must serve as a guide to the physiological needs of the body.

In their single choice experiments with adrenalectomized rats, Richter and Eckert ('38) report that in these animals, showing greatly increased appetite for sodium in the form of the lactate, there was no voluntary increase in their intake of chlorides of iron, magnesium, calcium, aluminum, potassium, or ammonium. In multiple choice experiments 11 rats were fed a low mineral diet and were given a choice of sodium chloride, sodium lactate, sodium phosphate, potassium chloride, calcium lactate, and water. Adrenalectomy produced an increased intake of the sodium solutions, but also a small increase in potassium chloride. The survival time was greatly increased by offering these salts. In another multiple choice experiment 6 rats were given a choice of a wider assortment of electrolytes: sodium chloride, sodium phosphate, sodium iodide, potassium chloride, ammonium sulfate, calcium lactate, and water. Adrenalectomy produced an increased appetite for all solutions except sodium iodide. The relative proportions of the increased intake of the different ions were practically the same as are found in normal blood serum. Sodium and chloride ions showed the greatest increase, potassium and calcium ions the smallest, and lactate and phosphate ions fell midway between. Daily injections of cortical extract caused the mineral appetite of these animals to return approximately to their normal level. The

latency period of the increase in sodium appetite averaged 3.3 d with a range of variation of 1 to 9 days. From these results authors concluded that the adrenal glands affect not only the me olism of sodium, but of most of the other electrolytes found normal blood serum.

Barelare and Richter ('38) found that, on the average, rats gested over twice as much sodium chloride (in a 3 per cent solut in the 10-day period after conception than in the 10-day period be conception, and over three times as much in the second half gestation. The sodium chloride intake fell back in the first 10 d postpartum to a level only slightly above the pregestational per But whether these data are significant as respects appetite dur pregnancy is not indicated since the consumption of other spe nutrients is not stated. Since pregnancy requires an increased f consumption, a demonstration of increased sodium chloride intak not important unless it is shown that the intake is peculiarly la in comparison to that of other nutrients.

Experiments with Thiamin Consumption as Affected Appetite.—Richter, Holt and Barelare, ('37) provided thiamin solution of the synthetic chloride) for rats on a diet deficient in nutrient, and apparently deficient in all components of the vitar B-complex, and found that the rats showed an overwhelming appe for it under such circumstances. One thiamin-deficient rat drank cc., or 5,500 International Units, in less than half an hour. The o as well as the taste of the vitamin aroused great interest in the and their craving for it was easily apparent. The animals found thiamin bottle at once even when there were as many as 12 contain filled with different foods or solutions, in the cage at the same ti Once the rats had started drinking, efforts to remove the bottle w met with fierce resistance, the bottle being held onto with paws a teeth. When riboflavin was offered under similar experimental c ditions the rats showed great interest in drinking the solution, by no means so intense a craving as for thiamin. In the case thiamin, the immediate craving shown by the animals, and th consumption of excessive amounts with greediness, seem to sh that in this instance, appetite does not depend upon the experie of a beneficial physiological effect upon its ingestion.

The possible relationship between appetite for specific nutrie and the needs for such factors merits particular attention. For t reason we have chosen to discuss some of the present data at so length in this chapter. It is necessary to point out, therefore, that above report on thiamin craving provides scarcely any informat at all on obvious questions relating to specificity of appetite. Ob ously the depraved appetite of the deficient rats caused them to ing

ar more thiamin than they could utilize in recovering from the eficiency, had there been only a simple deficiency of thiamin. It is ot reported whether, once the animals had ingested a reserve of iiamin, the appetite no longer made them greedy for it. Since several itamins, including thiamin and riboflavin, are available in pure form should be easy to quickly determine the extent that appetite, in its roadest sense, may be trusted to direct the ingestion of specific utrients in amounts required to promote health.

Self-selection of Foods by the Rat.—Richter, Holt and Barelare '38) reported that rats given a choice of purified casein, olive oil, extrose, sodium chloride, calcium lactate, dry yeast, and cod liver il, gained weight more rapidly than animals on a standard diet enerally regarded to be adequate. According to their calculations, on ie free choice plan the per cent of the total calories consumed in one roup were: protein 23, carbohydrate 56, and fat 21 per cent respec-vely. The animals did not all behave alike, however, since another eries selected protein 35, carbohydrate 12, and fat 53 per cent. Both roups appeared to be normal on the basis of growth, activity, and ex cycles. Animals given the above choice without cod liver oil eveloped symptoms of vitamin A deficiency and when given access cod liver oil showed appetite for it, resulting in disappearance of ie symptoms. When yeast was omitted, they developed a craving or it and ate the supplement with avidity when it was provided. he average free choice consumption of sodium chloride and of lcium was 0.12 and 0.18 gm. per day respectively. The consumption calcium was at least 3 times greater than has been found to be ptimal, as shown by various investigators. In these experiments the nly appreciable source of potassium, magnesium, iron, and copper as in the yeast. All of their rats were deficient in vitamin E, as own by half of a series of 8, which conceived, but did not deliver eir young. The results should not be regarded as conclusive until e experiments are repeated using purified materials that have been itically selected.

On the basis of these various studies by Richter and associates, it difficult to judge whether the data in any instance really demon-rate the development of specific appetite for indispensable nutrients hen they are deficient in the diet. However, the indirect evidence mineral consumption in parathyroidectomized and adrenalectom-ed rats is rather convincing. The role of appetite in the selection of od is an important field of investigation, but subtle variables are volved which require accuracy in technical procedures and an under-nding of present information on nutrition. Moreover, it should be lized that appetite in animals is far less complex than in humans. ie latter are constantly assailed by rationalizations based on false

notions, prejudices, and sales propaganda; thus rendering "instin in food selection, if it exists, practically without value.

Self-selection of Foods by Children.—Davis ('33, '34) repor experimental studies on self-selection feeding of newly-weaned infa and children. They were offered the choice of 35 articles, viz., be lamb, chicken, liver, brain, thymus and thyroid, sea fish, oyst whole wheat, oatmeal, barley, cornmeal, rye, bone marrow, bo jelly, eggs, certified raw milk, certified raw whole lactic milk, app oranges, bananas, pineapple, tomato, lettuce, cabbage, spinach, ca flower, peas, beets, carrots, turnips, potatoes, sea salt, and table s All of these articles were served during each day, in four mea Both kinds of milk, two kinds of cereal, either fruits or vegetabl and animal protein foods were served at every meal. Cod liver was given without considering the child's desire for it.

The results of these studies showed that children selected fro the list of simple natural foods a satisfying diet and maintain excellent appetites. They chose both the varieties and quantities the foods in a manner well within their digestive capacity, as co firmed by the marked gain in weight, their freedom from digesti disturbances, constipation, and anorexia. Analyses of their food recor showed that in spite of the apparent chaotic manner of selecti their intake of protein, carbohydrate, and fat bore an orderly relati to each other and did not differ widely in the group.

This study would have been of more significance concerni practical dietetics if the list of foods had included products such sugar, candy, nuts, cake, cookies, preserves, jellies, etc. The data off no suggestion of children's ability to select food wisely from a array containing the above items which are indeed common in th average household. When the choice must be made between natur foods such as those used by Davis, it is not surprising that childr thrive since the palatability of the foods would tend to encourage wide selection.

Appetite for Tea, Coffee, Alcohol, etc.—There seems to be n evidence that habits such as tea, coffee, mate, and alcohol drinki necessarily depend upon some degree of nutritional want. Certain many people who have available excellent food supplies form a appetite for one or another of these beverages, and miss them great if the supply is interrupted. These dietary habits merge in the nature into appetite for drugs obtained from tobacco, hashish, ka coca leaf and betel-nut chewing, opium smoking, etc. The physiologic incentive for masticating chewing gum should perhaps also me inquiry. The apparent preference of those used to corn bread ov wheat bread, and of the polished rice eater for boiled rice instea of our wheat bread, fall in the same category. The excessive eating

rn products or of polished rice exposes the individual to pellagra d beriberi. The fact that eating unpolished rice would protect the e eating Oriental from beriberi has not overcome his distaste for the milled product. The Newfoundland fisher folk could readily eat ely of fish livers, and greatly to their advantage as respects health, t experience shows that they do not avail themselves of that ad- ntage. Medical literature is replete with records of persons who luntarily restrict their diets because of the belief that certain foods, nerally found to be wholesome, do not agree with them. The thusiast meets with embarrassing facts when he tries to push too r the reliability of appetite as a guide to the selection of food. That gar eating tends to be habit-forming is well known. If, as seems t improbable, it will be established that sugar eating goes far to omote the establishment of *L. acidophilus* as the prevailing mouth ra, with dental decay as the result, the guidance of appetite will be own to be to our disadvantage.

Clay Eating.—Clay eating is practiced by people in many parts the world. To most people it appears inconceivable that anyone ould like it. Near Clarksville, Texas, the negroes eat a red clay. me eat it raw, and others bake it hard in the oven. They travel iles to get it (communication from Miss N. P. Morris, through the urtesy of Miss Ruth Van Deman). Possibly this practice points to me inorganic deficiency, yet all people in that region do not form e clay eating habit.

Case (cited by Kellogg, '23) of the Battle Creek Sanatorium, ob- rved that fuller's earth possesses laxative properties. Kellogg cites hers who have recommended taking a tablespoonful of washed olin before each meal as an excellent laxative. He also cites the port of a traveller in the South Pacific, who said that the natives certain islands wash down clay with copious drafts of coconut lk. Their reply to the question as to why they ate clay was that was good for them. Kellogg expresses the belief that clay eating is sed upon experience of its laxative properties.

Pica in Animals.—Pica is the general term for perverted appetite. is common among idiots, the insane, and children infested with rms. Chalk, string, cinders, papers, etc., are eaten. The cause, in any instances, is obscure. In animal husbandry pica is frequently a ious problem. The literature of veterinary medicine and of poultry sing is replete with reference to this form of abnormal appetite. us wool eating by sheep may be caused by faulty nutrition, but some instances it is due to the presence of irritating external para- es. Green ('25) cites a common type of pica. Penned sheep were l a faulty ration of maize endosperm and a minimal allowance of toclaved hay. Most of these sheep developed the wool picking

habit, nibbling at each other constantly, and many died of impact of the rumen with wool.

Green has reviewed the literature on the "licking disease" in cat which has been reported from various parts of Germany, Swed and France. It became a scourge in areas of reclaimed moorlands East Prussia. In the beginning there was reduced appetite, so degree of suppression of rumination, and frequent constipation. T was succeeded by depraved appetite, the animals eating mortar, ston wood, string, clothes, and their own excrement. These animals wh kept in stalls licked each other, the mangers, and walls, the licki continuing both day and night. The cause of the licking disease cattle has never been explained. It occurred so extensively as to calamitous to farmers in the affected regions. It was noted that h cut before flowering was harmless, but later cuttings were harmf The abnormality was confined largely to stall-fed animals, op grazing causing recovery, probably because of the selection of certa plants by the animals. Curiously enough horses did not develop t licking disease when fed the hays which produced it in cattle. this connection it is of interest to recall that horses seem never have developed the anemia due to cobalt deficiency, when confin to the same pastures on which cattle or sheep suffered fatal anem Comparable with wool picking is hair licking in colts and calves. general this abnormal habit develops when the animals are confin to food of poor quality. Apparently a counterpart of pica in domest animals is the licking habit which Orent and McCollum ('38) ha observed in rats deprived of potassium, and which thus far has n been described in any other specific deficiency of known nature.

Infantophagia.—Many wild animals, if alarmed over the safe of their young, destroy them soon after birth. That this may be manifestation of faulty nutrition is clearly shown in the freque occurrence of cannibalism in rats fed certain faulty diets. The exa cause of this has not been determined. Sows are notoriously given eating their new-born pigs under conditions of malnutrition, but t idea prevails among swine raisers that this pernicious practice is oft due to individual "innate" depravity.

Chick Vices.—One of the most perplexing problems involved chick raising is that of controlling cannibalism, feather picking, a toe picking. It is not known how far this may be the result of i proper feeding, which seems to be one cause. Careful observatio shows that too much light in the brooder, especially when the spa is overstocked, will tend to cause feather picking. This results cannibalism. The chicks are attracted by slight abnormalities su as a feather follicle which is congested, or by colored pin feathe which become highly visible in bright light. They peck at these objec

nd wound their companions, then the abnormal appearance of the irds encourages further attacks. Chicks will pick at any bright object, nd brightly lighted toes are a temptation to them. Once a toe is njured, chicks will persist in picking at it. Idleness and lack of exer-ise cause irritability and promote the development of vices. That unger, and possibly specific hunger for individual nutrients, may ause feather and toe picking, is shown by the fact that withholding ood too long from chicks tends to develop this abnormal behavior.

Egg eating is frequently practiced by hens which are fed inade-uate diets. McCollum has observed that hens on experimental diets esigned to determine whether birds could synthesize lecithin, de-eloped the habit of egg eating so badly that they had to be carefully vatched in order to secure the eggs immediately after they were aid, otherwise they were broken and eaten by the hens. Champions f the reliability of appetite as a guide to food consumption should xplain why a flock of chicks will fill their crops with grains of rock alt where an ice cream freezer has been emptied and, as a conse-uence, quickly pass to their avian reward.

Iron Eating by Cattle.—In many parts of the United States cows at nails and wire. No extensive study seems to have been made to de-ermine the extent of abnormal craving of iron by cattle. A typical ex-perience was reported to McCollum by a physician in Pennsylvania who owned a valuable dairy herd and who had suffered considerable osses as the result of the cows eating nails and pieces of iron wire. Perforations of the third stomach caused peritonitis. The physician nade many observations on his cows and found that they would letect bits of wood, porcelain, glass, copper, zinc, pebbles, etc., placed n the mangers and would invariably discard them. On the other hand, vhen they found a nail or piece of wire, even a foot or more in ength, they would treasure it and chew on it, eventually swallowing t. The cows would lick rusty iron objects placed in their way out f doors, but would not lick polished iron. They did not lick copper r other metals which were available. Veterinarians are familiar with he finding of a quart or more of nails in the third stomachs of attle. It is not known whether this habit is an expression of iron leficiency. Apparently in many instances it is not, since it occurs in ows which are not anemic. Further study of this problem should e made to determine whether fortifying the ration with soluble iron alts would do away with the habit. Such an investigation would be f interest and importance since nail and wire eating cause consider-ble mortality among cattle in America.

Other Instances of Abnormal Appetite.—Some persons experi-nce an inordinate craving for food (bulimia), as in the case of preg-ant women who tend to long for certain foods not easily available.

Also, in certain neurotic conditions there is an abnormal craving
food. A voracious appetite has been noted by Howard ('22) am
the symptoms of hyperthyroidism. It is associated with disturba
of the tonic innervation of the alimentary tract. Bulimia is also s
in diabetic patients. In hyperthyroidism and diabetes, excessive cr
ing for food probably represents an expression of actual need
nutriment, since in the latter much carbohydrate food is leak
away unused in the form of sugar in the urine.

Opposed to abnormal craving for food is anorexia, or loss of ap
tite. In this condition there are no manifestations of hunger althou
the body may be in dire need of nourishment. Anorexia has be
noted by several observers in deficiency of thiamin. It also occurs
gastritis, neurasthenia, and various other conditions. A positive loa
ing for food is characteristic in certain cases of hysteria.

Whether the arsenic eating practiced by women in Hungary a
the Tyrol for whitening their complexions and by men for increasi
physical endurance represents a perverted appetite is not clear, sin
it is done with a definite object in view.

In chlorosis, a form of anemia most common in young wom
and characterized by marked reduction of the hemoglobin content
the blood, but with a nearly normal red cell count, it is said that n
infrequently there develops a craving for sour-tasting foods.

These are only a few of the different manifestations of abnorm
appetite. It is possible to conclude that specific dietary deficiencie
probably produce cravings for food, which, if allowed to be volu
tarily satisfied, would tend, in some instances, to correct the conditio
But there is a preponderance of evidence that in humans, appeti
generally is not a reliable guide to physiologic needs.

REFERENCES

Cannon (1934). "Bodily Changes in Pain, Hunger, Fear and Rage. 2nd ed., D. Appleton-Century Co., New York.

Carlson (1919). "The Control of Hunger in Health and Disease." Un versity of Chicago Press, Chicago.

Morgulis (1923). "Fasting and Undernutrition." E. P. Dutton & Co New York.

Shelling (1932). J. Biol. Chem., *96,* 195.

Evvard (1929). Iowa Agric. Exper. Sta. Bull. No. 118.

Osborne and Mendel (1915). J. Biol. Chem., *20,* 351.

Osborne and Mendel (1918). J. Biol. Chem., *35,* 19.

Richter (1936). Am. J. Physiol., *115,* 155.

Richter and Eckert (1937). Endocrinology, *21,* 50.

Richter and Eckert (1938). Endocrinology, *22,* 214.

Barelare and Richter (1938). Am. J. Physiol., *121,* 185.

ITER, HOLT and BARELARE (1937). Science, *86*, 354.
ITER, HOLT and BARELARE (1938). Personal communication.
IS (1933). Am. J. Dis. Child., *46*, 743; id. (1934), J. Am. Dental SSOC., *21*, 636.
LOGG (1923). "The New Dietetics." The Modern Medicine Publish-g Co., Battle Creek, Mich.
EN (1925). Physiol. Rev., *5*, 336.
NT and MCCOLLUM (1938). Unpublished data.
VARD (1922). Endocrinology and Metabolism. Edited by Barker. I, 331, . Appleton & Co., New York.

CHAPTER

XXVI

Dietary Habits of Man in Different Parts of the World

IN THE POPULAR literature relating to healthful living on infrequently meets with statements which convey the view uncivilized and primitive peoples generally live more nearly i cord with "Nature's laws," and have better health than do p living under modern conditions, who have more comfortable sh a more varied diet, and far greater assurance of a regular supp foods than was customary in many inhabited regions of the pa today in the more isolated areas of the world. Careful scrutiny o facts reveal, however, that peoples living under primitive condi now and in the past, have led more or less precarious existenc respects exposure and food supplies. Far more generally their have been too restricted as to source and have been too unvar As a consequence the people have tended to be malnourished. U hard conditions of life only the physically best-endowed survive.

That people in regions strongly contrasting as respects cli and soil have survived on diets of widely different natures is not construed as indicating that it makes little difference what w so long as we have enough to meet our energy needs. As disc has succeeded discovery in the science of nutrition, attention has increasingly directed toward appraising the quality of human taries in every country. In our present favored position as res knowledge of the needs of the human body for individual nutr it is of great interest and importance to survey the different typ diets which have sustained man at various times and in various p

The diet of all human kind previous to the modern era of s transportation by land and sea depended upon what could be se locally, either by hunting, fishing, foraging for wild products, re of domesticated animals, or the cultivation of plants. Climate

il determined success in any of these ventures. Even today large umbers of people are entirely dependent upon local sources of food nce they have no opportunity to secure other foodstuffs through ade. In this chapter the effects on human nutrition of climate, soil, nd natural resources will be briefly considered.

Dietary Habits of Early Egyptians.—It is profitable to begin is discussion with some consideration of the dietary habits of the rly Egyptians since agricultural pursuits, obviously of basic impornce in the provision of food for large populations, are reputed to ave had their origin in the region of the Nile. Ruffer ('21) states at the Egyptians were called "eaters of bread," and to them, as to e Hebrews, bread was synonymous with food. It was made from elt (which is a kind of wheat), bearded wheat, or barley. Cereals ere cultivated from early times. Flour was prepared in the preynastic period by grinding grain between two stones or pounding in a mortar. Ruffer comments that the teeth of the soldiers must ave been severely tried by their daily ration of 4 pounds of bread ecause of its coarse texture. Lentils, beans, artichokes, asparagus, eet root, and cabbages were doubtless cooked, but onions, cucumers, garlic, radishes, and turnips were eaten raw. The chief fruits f the Egyptians were grapes, figs, dates, and dûm palm, pomeranates, melons, carobs, olives, apricots, and the seeds of marshy lants. Although the wealthy had large fruit gardens, Egypt has ever been an extensive fruit-growing country. Egyptians of the poorer lass, like the fellah, probably tasted little fruit except dates and elons.

The diet was not wholly vegetarian, for the predynastic refuse eaps of El Toukh have yielded many remnants of animals which rved as food. Cattle were kept as far back as Egyptian civilization an be traced, and many wild animals and birds were kept in capvity and artificially fattened. The Nile and its canals were inexaustible sources of fish. Salted and pickled fish were favorite articles food. Pliny states that the ancient Egyptians were accustomed to ew the stems of the papyrus rush, the juice of which was swallowed. his plant has long since been far less abundant than in early times ving to the extension of land utilization.

The Diet in Cold Regions.—Since in the Far North there is little no exposed soil in most places, and low temperatures prevent any nd of agriculture, man's food is perforce restricted to fish, mamals, and birds which derive nutriment from the water life, together ith such land animals as the reindeer, caribou, and musk-ox, that e sustained by the sparsely distributed lichens which they are able cat. The dwellers in these regions are occupied almost constantly, hen weather permits outdoor activity, with the quest for food.

Periods of scarcity and privation are not infrequent and many Eski have died of starvation. They work hard when conditions per and, like hungry animals, eat to satiety when food is abundant. T survive the climate because they wear effective clothing which m tains essentially a subtropical environment about the clothed pa The only actual stimulation of metabolism is caused by breathing c air when out of doors in winter and by their consumption of a hi protein diet. Their liking for fats and oils is the result of custom hunger, and not of climate.

Formerly it was customary to dismiss the Eskimo diet with statement that it consisted of meat and fat. We now distinguish tween muscle meats, incomplete nutritionally in a number of spects, and glandular organs which supply, when eaten raw, th essentials lacking in muscle meats. Eskimos, and the sparse Ind population at the southern end of South America, survive beca they eat all parts of the animals which serve them as food. M could not survive and continue to propagate the race on a diet muscle meats. Fish, seal, walrus, birds, and eggs, and an occasio whale practically constitute the limit of variety in food for the m isolated Eskimos. Those further south supplement these foods wi the fish and organs of reindeer. About the only vegetable food ava able is the half digested moss of the rumen of the reindeer, whi they are said to relish greatly. Like the carnivorous animals whi subsist on a similar diet, the carnivorous peoples are healthy a capable of considerable endurance.

That the Eskimo is at some disadvantage because of his carniv rous diet is shown by investigations of Heinbecker ('28, '31, '32), wh found the basal metabolism of Polar and Baffin Eskimos to be co siderably higher than of persons living in temperate regions, doub lessly caused by excessive protein consumption. He found their carb hydrate tolerance to be fairly high, and the non-protein nitrog content of their blood was essentially normal notwithstanding the high-protein diet. Rabinowitch and Smith ('36) also found hig basal metabolic rates in Eskimos from Hudson Bay north to Devo and Ellesmere Islands, but their results differ from those of Hei becker in that they found abnormally high non-protein nitrogen in t blood of these people. Rabinowitch also found some cases of arteri sclerosis. The plasma chloride content was higher than the avera for peoples elsewhere, and in some subjects the renal threshold f glucose was above average values. He regards the high non-prote nitrogen of the blood as due to the high protein diet and not to in pairment of kidney functions. He cites data collected by Krogh a Krogh, according to which the average daily diet of these Eskim consists of approximately 54 gm. carbohydrate, 135 gm. fat, and 2

m. protein. Also, the observation was made that fat is not regarded s a delicacy.

The Diet in Warm Regions.—In large areas of the world's warm egions the principal food is rice, hence our discussion here is con- erned largely with this food. But we must distinguish between rice- rowing regions and those where it is not grown. Rice is the cereal f the swamp and is grown largely in delta regions in India, China, nd certain other areas in the Orient. But in the Philippines, Java, apan, Ceylon, and several other regions it is produced on terraced illsides. In both lowlands and terraced lands rice-growing requires n extraordinary amount of human labor. Also, it requires a definite ind of political and social organization. In lands such as India, hina, Japan, and Java, the human population presses so hard on ie agricultural resources that there is little food available for ani- als, and food is grown almost entirely by human labor. Smith ('19) ives an absorbing account of this form of agriculture.

Rice is deficient in protein, and since it is generally eaten after olishing, which removes the bran layer and the germ, is far less utritious than the whole grain, owing to the loss of B-complex tamins and some minerals as well as protein. In China and Japan ie chief dependence for a protein supplement is the soy bean, which ntains about three times as much protein as does rice, and twice at of wheat. Its protein has a lower supplementary or improving lue for rice proteins than is true of protein of milk, meats, or gs, but its inclusion in the diet of the rice-eater is extremely im- rtant.

Many centuries ago the Japanese and Chinese practiced fish-grow- g in ponds to supplement the supply from rivers and the ocean. his is one of the easiest and cheapest methods for producing flesh ods. Also, it conserves land and utilizes material in water which herwise would be wasted. In the densely populated regions of the ce-growing areas of China the only animals produced in consider- le numbers are pigs and poultry, since they are foragers and sustain emselves on foods which are not suitable for man. The poor coolie pulation is nourished largely on rice, soy bean curd, and a little rk and fish. Among those better off economically, eggs, meats, fish, uits, and cereals other than rice are more prominent in the diet. nce the extremely poor constitute a great majority of the popula- n, simplicity of diet, with excessive rice eating, characterizes all the congested hot swampy and delta regions, and large hill areas Oriental countries. Wang ('20) and Wu ('27) have discussed the equacy of the Chinese diet.

The prevalence of beriberi in rice-eating countries is indisputable idence of the inadequacy of the diet of scores of millions of people.

Commenting on the nutritional status of the Chinese, Wu (l.c.) st "The Chinese are small in stature compared with Americans English. They live shorter lives with higher mortality both an adults and infants. They possess low vital resistance, as evidence the prevalence of such diseases as trachoma and tuberculosis. T are over-peaceful, non-persevering, non-progressive, non-enterpri and are easily contented with the environment in which they themselves. Are these qualities inherited from the ancient Chinese came to Eastern Asia thousands of years ago, or are they the resu malnutrition continued from generation to generation? There is siderable evidence that the latter is the case." One is constraine point out that when a country is grossly over-populated, and the st gle for existence is extremely great, the incentive to effort is lowe since the prospect of reward for effort sinks almost to zero, except the favored few. This applies to all the congested regions of Orie countries. It is stated by a Committee working under the ausp of the Health Section of the League of Nations ('37) that there large areas in Asia where 90 per cent or more of the extren small family budget is spent for food. Obviously any attempt improve the nutrition of people in these regions must begin w an improvement in their economic situation.

The Cassava Eaters of Java.—In Java there is a region inhab by perhaps 3 to 4 million people, where the soil is almost pure ch and the only plant of agricultural value which can be grown cassava, the starch from which constitutes our tapioca. These pe are in the worst extremity economically. They eat excessively of cass roots and suffer severe malnutrition. The cassava-eater experien a peculiar craving, a kind of perverted appetite, for the djenkol be the product of an indigenous tree. This bean is poisonous, an single bean causes some degree of illness, but the people persist eating it because the bean affords a kind of misery differing fr and preferable to deficiencies from cassava.

A Problem of Certain Oriental Colonies.—Java affords an cellent example of the plight of certain Oriental peoples. As a col under Dutch administration the situation of its native inhabita has undergone great changes. Under primitive conditions it ha considerable population, which was limited to the more favored ar Under Dutch dominion much land was brought under cultivat to provide sugar, rubber, coffee, tea, and quinine, largely for exp Such trade is the factor that makes it worth while for Europe to administer colonies in warm countries. They extend to the li the growing of things which cannot be used as food and ship th away. But such agriculture demands laborers, and a numerous po lation is desirable. The Dutch are a highly capable people and in

duced measures for the control of malaria, dysentery, yaws, beriberi, and other diseases, thus lowering significantly the death rate. The consequence of these health measures, together with considerable improvement in the economic status of a part of the natives, was a seven-fold increase in the population within one century. With the present dense population it is economically impossible to improve the dietary of the Javanese, and were it to be done, it would be at once reflected in a decreased infant mortality, an extension of the child-bearing age of women, and an increase in the span of life. With a population which devotes itself whole-heartedly to procreation, as do the Javanese, the Island would speedily become a liability instead of a colonial asset. In the two chief hospitals in Java, 24 per cent of the 5500 deaths reported during the years 1919 and 1920 were ascribed to starvation. The hospital records represent but a very small fraction of the total illness of natives in this land. The death rate from starvation is appalling.

The Primitive Forest Peoples.—There are said to be about 85 million African people living in the forest area between the Soudan Desert and the Kalahari Desert. These, like the dwellers in Equatorial America, in the jungle areas, and similar groups in the East Indies and Indo-China, all dwell where the rainfall is heavy, the air is hot and moist, and where vegetation grows so rankly that it is almost impossible for man to practice agriculture. In the rice fields of the world much of the work is done by hand, but some aid is secured through the use of oxen and water buffaloes in dragging the ploughs through the muck. In the case of the peoples we are now considering, animals have never been used in agriculture. Water buffaloes withstand tropical heat but they are useful only in rice cultivation since under such conditions the fields are covered with water and mud. The Aryan developed from savagery through the animal-tending or pastoral stage, but these forest peoples developed a hand agriculture. The Panama farmer, who practices this simple form of agriculture has but two implements, the machete to cut brushes and weeds and a sharp stick to make holes for the seed. The natives of Panama depend almost exclusively on such starchy roots as the sweet potato, yam, taro, and manioc (cassava). In addition they eat caladium, bananas, sugar cane, and maize. Apparently the economic and physical condition of such peoples has remained essentially stationary throughout the ages. The climate is enervating, intestinal parasites abound, and insects torment the inhabitants and transmit diseases which impair health. There is no reward for industry, and they work only as they are goaded by necessity. It is not possible to sort out the effects of inadequate diet from other debilitating factors in their environment, as respects their influence on health.

The Diet of the Mambuti, the East Congo Pigmies.—Van Bergh ('21) who visited the Pigmies of the eastern edge of the Co Forest, states that their food consists of a considerable variety cluding the manioc, and other roots, which they eat raw althou these foods are difficult to digest. Nuts, wild fruits, and tender sh of plants are also eaten. These people are capable hunters, and many wild birds and small animals and occasionally an elephant food. They also eat ants and caterpillars. There is no note of t being frequently short of food and with their varied, mixed diet seems strange that their stature should be so small.

Human Food in the Driest Regions.—In the desert borderla of North and South Africa, Arabia, and Asia, people can subsist o through the conversion of a scanty pasturage into food by me of flocks and herds. These peoples are represented in the extreme no by the Lapps, where cold, mountainous lands compel a similar m of life, and in more southerly regions by the Mongolian nom Kirghiz, Turkoman, Arab, and Taureg. Depending upon conditi they care for reindeer, cattle, sheep, goats, horses, and camels. though they possess many animals in proportion to the human po lation, they do not, as a rule, eat much meat. The reason for this that they cannot afford to kill female animals so long as they are value for breeding, and such of their wants as are to be obtain only through commerce with neighboring peoples must be purcha generally only by the sale of young male animals and skins. Su peoples subsist principally on milk and milk products. Ordinari milk is soured readily by mixing it in containers with a small amou of sour milk, which causes it to undergo lactic acid fermentati promptly. The exceedingly restricted diet is made more tolera by the use of some cheese. Of course, some other foods are alwa to be had. The Lapp, for example, takes advantage of any opp tunity to secure other foods. Some of the Lapps are fishermen, b they fare less well than the reindeer keepers. All Lapps secure so green food in the form of a few succulent plants found in the mou tains and they also buy a little food, mostly cereal, from the neig boring people.

The desert Arab grows tired of his almost constant diet of so milk, but there is nothing he can do about it. He always gets so barley or wheat which is made into bread, and generally some da as well as small additions of other vegetable foods, but these foo supplemented occasionally with a little meat, are all that he has, a they sustain him in health so far as food determines health.

The Kirghiz nomads, who follow their flocks and herds of hors yaks, and sheep in the lowlands and high plateaus of Central A depend more for sustenance on milk, cheese, and butter, than a

er people. They hunger for bread, which is very scarce, and have means of varying their diet. Yet, with the slight amount of supplentation provided by other foods, their diet is apparently come, in the sense that it provides everything that is essential for siological well-being, since the Kirghiz are capable of great endure.

The Hottentots of the Kalahari Desert region live according to ntially the same plan. They dwell in the region of sparse grass on border of the desert and raise cattle and sheep. Their food is cipally milk and cheese, with some meat, and a small supplement egetable foods. Huntington ('27) has provided a fascinating deption of the effects of geographic environment on their habits of He states that the Hottentot man has been described as indolent, he is capable of great exertion. It appears that when people de- for their livelihood on the keeping of flocks and herds, the men st be kept in a rested condition, in order that they may meet essfully the emergencies which not infrequently arise in retrieving animals that have strayed too far, or have been stampeded by a animal or bewildered by a storm. Huntington points out that such emergencies a man, already wearied with other work or kened by poor diet, would be unequal to the task before him.

These brief examples must suffice as illustrations of the food ts not only of the peoples of the dry regions of the world, but of the keeper of flocks and herds in colder regions. The point e emphasized is that these are the great milk users of the world. ir mode of life necessitates reasonably good health and endurance these their diets sustain despite their monotonous and unappetizqualities. An appreciation of the mode of life among Pastoral les may be obtained from the Old Testament since the conditions ribed there still exist.

The Diet of Hunting Peoples.—The most typical hunting peoples oday are the Bushmen of the Kalahari Desert, who have been ed by their neighbors into a region so dry that farming and ing are impossible, the Samoyedes of Northern Siberia and, e recent past, the Indians of the Great Plains of Central North rica.

he Bushmen live almost entirely by hunting, but their women, by ng, obtain certain succulent herbs which grow in the desert s. Their chief source of food is the antelope, which, being very , requires great intelligence and skill to secure. These they stalk shoot with arrows, generally poisoned, but at times they chase on foot, and secure them only after both men and beast are cally exhausted. It is obvious, therefore, that their nearly carous diet sustains good physical development and endurance.

But life is exceedingly hard for the Bushmen and only th strongest and persevering survive.

The Samoyedes of the lower course of the river Ob h domestic animals and subsist entirely by hunting and fishing have a low order of culture, but, like the Eskimo, their diet, it is to secure in sufficient amount, sustains them under diffic ditions as respects climate and the necessity for hard work. N compels them to eat everything available, and affords an exam the significance of the glandular organs as supplements to a muscle meats and fish. Food is at such a premium that the eat the flesh of carnivorous animals, including the wolf, al such flesh is of bad flavor.

The Diet of American Indians of the Plains.—The Indian inhabited the plains east of the Rocky Mountains were in measure a carnivorous people, since they subsisted mainly flesh of the bison, which roamed in enormous herds from Te Southern Canada. Catlin (1861) visited a Mandan tribe in 183 states that in addition to buffalo meat, which they secured in su ous supplies in their hunts, these Indians ate some wild frui berries; and some tribes cultivated corn, pumpkins, and squas kind of wild turnip, called "pomme blanche" by the French much sought after by migratory Indians and was eaten ra highly was this plant valued it is said that the route taken in foll the buffalo was often determined by the localities where this was to be found. Wild plants and trees furnished some vari season, of plums, choke cherries, grapes, elderberries, raspb strawberries, etc. "Indian potato" and falcata beans were also The latter produced two products: pods containing small bea which little attention was given and large underground beans were sought after because of their agreeable taste. These beans collected by meadow mice into hoards of a pint or more, and secured by the Indians from this source. For winter use the Indians dried buffalo meat in strips over a smoke fire to prese during warm weather and to keep off the flies, the maggots which would spoil the meat. This meat was pounded into a powder and mixed with fat to form pemican. The fat most prized was that from marrow bones, but the body fat wa used for this purpose. Pemmican was stored in bladders, or i bags.

Wherever conditions made agriculture possible, the primiti dians grew corn, beans, pumpkins, and squashes, to supplem game on which they principally depended. This was especiall of the Indians of the Southwest, and certain parts of the So States, where some tribes were more settled agriculturalists. Alt

tistics are wanting, there is abundant testimony that the teeth of hunting Indians were essentially free from caries. Catlin (l.c.) amined a large number of skulls of Sioux Indians, which it was ir custom to collect and preserve in circles on the ground after the rial scaffolds gave way. He says: "I was forcibly struck with the most incredibly small proportion of crania of children; and even ore so, in the exceptional completeness and soundness of their beauti- sets of teeth, of all ages, which were scrupulously kept together, the lower jaws being attached to the other bones of the head. The th arrange themselves very regularly as the keys of a piano. Their th do not decay but preserve their soundness and enamel and wers of mastication to old age." Catlin, when in company with a rty of 14 Iowa Indians visiting England, states: "The Chief informed that one of the most striking peculiarities which all Indian tribes scovered amongst the white people was the derangement and ab- nce of their teeth which they believed were destroyed by the number lies passing over them." Needless to say this attractive hypothesis not supported by modern research, but no one can deny that, for merican Indians living under the conditions of that period, it was a ausible deduction.

The Indians of the Southwest of today differ much in respect to rtain articles which they eat, for, in general, they all subsist too rgely upon maize, beans, and coffee. They generally have access to eat of some kind: beef, mutton, and wild game. Some eat field mice d prairie-dogs, and others, as the Apache, eat considerable amounts cactus fruits. At the present time some of the Apache move in the ring to areas where the giant cacti (Cereus giganteos) grow abun- ntly and remain there for two or three months until the last of the it has been matured and gathered. Numerous seeds of wild plants also gathered and eaten. It seems unwarranted to attempt any praisal of the adequacy of the diets of these Indians in relation to ir health, since they are generally poor and are sometimes in want. rdlička ('08) has written an extensive account of the foods of dians in that region 30 years ago. He states that, on the whole, health of the Southwestern and North Mexico non-civilized In- ns is superior to that of whites living in larger communities. He gards the advantage of these Indians to lie principally in their ater freedom from those various morbid conditions that arise rough poor inheritance. The only genetic disadvantage of the Indian, states, consists in a possibly weaker resistance to a few of the ntagions.

The Diet of the Reservation Indian.—As wards of the Govern- nt the reservation Indians have suffered greatly from tuberculosis, d from epidemics of various kinds, to a greater extent than has the

general population. That their diet has been inadequate seems h probable from their health record and also because of the ma increase in the incidence of dental caries since they became depen In the absence of knowledge concerning the properties of foods of the nutritive requirements for health, it is not surprising that i past the rations supplied to reservation Indians provided exce amounts of milled cereal products, syrup, molasses, sugar, and ca goods such as peas, corn, and tomatoes, which had little su mental value as respects each other. These Indians require al above all other needs a better food supply.

The Diet of Primitive Pacific Islanders.—We have an exce account of primitive life of the Fiji Islanders from the book wr in 1859 by the Rev. Mr. William Cross and the Rev. Mr. D Cargill (1859), English missionaries, who, with their families wer Fiji in 1834 and remained there for many years. The natives ra large quantities of taro, a variety of the elephant's ear or dasheen composition it does not differ much from such tubers as the po sweet potato, yam or cassava. They also grew sweet potatoes, bana plantains, and sugar cane. Wild trees furnished breadfruit and c nuts. They made bread of taro, yam, arrowroot, breadfruit, or bana An inferior bread was made with the fruit of the mangrove arum, and the seed pits of dawa and kaveka. The bread was ba in excavated pits. The less desirable foods were eaten following g which swept away the plants from cultivated land. Other wild p ucts available in abundance were the bulou, which resembles an potato, weighing from 1 to 8 pounds; the yaka, a creeper with a very like licorice; wild tumeric; the root and fruit of kaili, a climb plant; two kinds of tomatoes; the leaves of bele which were e as greens; two kinds of nuts, agolago and vutu. The latter tastes unlike the peanut. There were also wild plums, wild figs, and fruit of the pandanus. There were men who devoted themselve fishing, and an abundance of fish were obtained. Turtle fishing w regular practice and turtles and their eggs formed a staple articl diet. Bicho-de-mar, which is the sea slug (also sea cucumber) v abundant and were eaten frequently. They were also collected barter. Almost every kind of inhabitant of a coral reef, inclu molluscs, shrimps, and other creatures were eaten. They also m several kinds of warm infusions from aromatic grasses and leaves drinking and they drank the juices of the ti-root, sugar cane, coconut. The chiefs and other dignitaries were addicted to a dr called yaqona, which was made from the root of *Piper methystic* It had narcotic properties. The drink was prepared by chewing root, an operation performed by young men. Water was added the chewed root, the solid particles were squeezed out, and the lic

ined. The drink was stupefying. As a rule women did not drink ona.

It will be seen that the primitive Fijians had a remarkably varied excellent diet. All who have written of them have remarked upon ir physical perfection. Their teeth were of excellent structure, and erally remained free from decay. This is of particular interest ause of their custom of eating freely of cooked starches, capable fermentation to acid, and their consumption of the sweet juice of ar cane. It is obvious that cooked starch, though pasty and capable dhering to the teeth in sheltered parts, may be eaten daily without disposing to tooth decay.

The Sequelae of Foreign Invasion of Pacific Islands on the alth of the Natives.—Cilento ('28) states that the Western Islands the Territory of New Guinea formerly supported a numerous popu- on of vigorous people, but commercial grants made to foreign mpanies during the past fifty years have deprived the natives of best agricultural lands and have forced them to subsist mainly on swamp taro, yams, crude sago, bread fruit, and bananas. The piriting influence of pure food, malaria, and tuberculosis have re- ced their efforts in fishing, and although some fish, shark flesh, eons, parrots and lizards are eaten, their diet is very poor as com- red with that before foreign invasion. It is deficient as respects pro- ns, vitamins, and mineral elements. Dental defects are very com- n, and Cilento found the degree of dental deficiency to vary in ferent islands directly with the insufficiency of food as judged by tritional quality. Loose, blackened, and filthy teeth are extremely mmon among these islanders. It is not possible to determine how the prevalence of disease as opposed to poor diet is responsible for sent conditions.

The Island of Tristan da Cunha.—The Island of Tristan da nha lies in the South Atlantic, about 2000 miles west of the Cape Good Hope, and about 4000 miles northeast of Cape Horn. Mar- ll ('26), surgeon of the *Discovery,* visited the Island in 1926. The pulation consisted of 75 males and 66 females, of whom 69 were lts, 21 were aged 14-21, and 51 were under 14 years of age. Three men were over 80, and the oldest man was 68 years old. The island isited by ships only at long intervals, and the natives are dependent n their local food supply. The islanders possess cattle, sheep, pigs, keys, chickens, and geese. Potatoes are the principal source of bohydrates. Onions, pumpkins, turnips, carrots, beets, and leeks grown in lesser quantities. Fish, although plentiful, are not popu- as food. The natives have an abundance of mollymauk eggs, and ng mollymauks are consumed in great numbers. Milk is always ndant and is a staple article of diet.

The islanders do not have cereals and hence do not eat b They have only insignificant amounts of sugar, tea, coffee, b jam, and marmalade. Of special interest is the high degree of fre of the islanders from dental defects. Marshall examined the tee more than one-third of the population and reported results as sented in Chapter XXVII.

It was Marshall's view that, had the entire population bee amined, the incidence of dental disease would have been found lower. The teeth examined were almost without exception regular well shaped. Although in no case did anyone admit cleaning h her teeth oftener than once a week, and then with finger and s the teeth were clean and remarkably free from salivary calculi. Tri islanders afford another example, therefore, of a people who practi never clean their teeth, who subsist in large measure on milk, pota eggs, and meats, and who remain remarkably free from tooth de The islanders, being isolated most of the time, have few epide of disease, but complain that after ships call there epidemics of c run through the island, lasting about three weeks.

The Diet of the Japanese.—The food supplies of the Japa have been most thoroughly described by Grey ('28). As is known, polished rice constitutes the principal food. This prod which is lacking in all vitamins, and is extremely poor in min elements and low in proteins, may be regarded as an energy-yield food only. The Japanese also eat considerable amounts of potat sweet potatoes, yams, taro, arrowhead, kikyo root, lily root, lo and other root vegetables, which, while of greater value as sou of vitamins and minerals, are poor protein supplementary foods w combined with polished rice. The people are saved from the m serious forms of malnutrition, except beriberi, by their liberal c sumption of soy bean products, which are very rich in proteins, unfortunately of relatively low biological value, and by their fa high consumption of fish, and of green vegetables. Soy beans, h ever, are not to be rated as an excellent supplementary food for r tubers, and root vegetables. Fruit eating is rather general. The c standing shortage of the more important foods in Japan are milk flesh foods.

In considerable measure the small stature of the Japanese is pr ably referable to inadequacy of their diet. Kanzaki ('21) found J anese children of all ages, born and reared on the American Pac Coast, to be larger than children of corresponding age in Japan.

The Diet of the Chinese.—Wang (l.c.) and Wu (l.c.) have scribed the diet of the Chinese. They have a wide variety of foo including eggs, meat, fish, fruit, cereals, and numerous vegetab In the southern part of China rice is the staple cereal, but in the no

part there are many people who never eat rice, but eat wheat ead. Pork is almost universally eaten, and is so common that the e well-to-do refrain from eating it. Lamb is said to be abundant, beef is seldom eaten. The quantity of meat consumed is, on the le, small, being used largely for adding flavor to vegetable foods. , crabs, shrimps, lobsters, and oysters are extensively used. The nese are less fastidious about eating brain, blood, spinal cord, and , than are Americans. Chickens, ducks, and geese are produced food in large numbers, and pigeons, turkeys, and pheasants are commonly available. Eggs of hens, ducks, and pigeons, enter in form or another into many Chinese dishes. Eggs are preserved t least three ways: hulidan, or salted eggs, dsaudan or fermented , and pidan or Chinese old eggs.

Vegetables are eaten more freely by the Chinese than by Ameri-. Spinach, cabbage, potatoes, onions, radishes, radish leaves, shep-d's purse, bamboo sprouts, and several kinds of sea weeds, are mon. The late Mr. Holden, a missionary, told one of the authors V. McC.) of the interest shown by the Chinese in young alfalfa es as human food, when he introduced the plant on the Mission n.

The Chinese cultivate no less than 20 varieties of soy beans. Adolph a, '25b) has discussed the Chinese diet, and particularly the uses le of soy beans. This bean is perhaps their main reliance for pro-. In the northern parts of China wheat, maize, and millet are ex-ively eaten. Of special importance is the practice of sprouting ns and beans before using them as food. During germination rbic acid is formed in relatively large amounts, and there is reason believe that sprouting increases the availability of nutrients in d seeds. Buck ('37) has given an extensive account of food pro-ion in China.

On the whole, Chinese peoples have a satisfactory diet as respects ity but even in the best times food is scanty in China because of population. As in other countries, the very poor, who are numer-subsist too largely upon cereal products and beans. Hawks ('31-has reviewed the literature relating to growth of Chinese children China, Hawaii, and Chicago. It appears from data available that nese children are taller for their weights than American children. nese peoples represent a number of different racial strains, rather a homogeneous stock. It is unprofitable to attempt an interpreta-of the adequacy of the Chinese diet as compared with that of r countries on the basis of size of children or adults. It is evident when the economic situation of the individual is such as to make ible the provision of an adequate diet, the composition of the nese diet approaches full adequacy. But the frequent famines,

especially in the Yellow River Valley, have from time immem been accompanied by epidemics of cholera and typhus, and nc frequently diseases due to dietary deficiencies have been a sore tion.

The Nutrition Problem of Young Children in Oriental C tries.—From what has been said it is apparent that the milk su of Oriental countries generally is quite inadequate. This is particu important in the nutrition of infants and young children. In all graphic areas where the milk of animals is available it has alv been the custom to transfer the child after weaning to a diet in w milk is prominent. The Oriental types of diet are not suitable young children, and there is a period following cessation of br feeding when the child's health is jeopardized by feeding it on v the adult population eats with impunity. A diet of cereals, vegetal and flesh foods will not support optimum growth in the very you and needs to be supplemented with milk. If this is not done the c tends to suffer a period of retarded development, and may exp ence considerable digestive disturbance. To meet this problem it long been the custom in Oriental countries to breast-feed infants children much longer than in Western countries where an abund milk supply is available. Appleton (private communication) sta that she could easily recognize in a group of Chinese children who had been given canned milk.

The Diet of Labrador.—Appleton ('21) described the diet of natives of Labrador. Its approximate composition is set forth in following table:

Bolted wheat flour	1.25 to 1.5 bbls. per person per year	
Salt meats, pork or beef	1 to 2 bbls.	for a family of eight
Salt codfish	200 to 400 lbs.	
Salt herring	1 to 3 bbls.	
Molasses	160 gals.	
Potatoes	1 to 2 bbls.	
Rutabagas	1 to 2 bbls.	
Dried peas	20 to 40 gals.	
Raisins	10 to 20 lbs.	
Butter substitute	80 to 160 lbs.	
Condensed or evap. milk	from a few tins to 2 cases	
Tea	20 to 40 lbs.	

Fresh trout, cod, and salmon are caught only during the su mer. In favorable seasons enough cabbage is grown to last u November. Rice, onions, and dried beans are used to a very limi extent. The only fresh fruits eaten are partridge berries, and a li wild berry called baked apple. Dock and Alexander plant, which semble spinach and parsley, respectively, could be used as greens

nsiderable advantage, but they are little eaten. Thus the appetite es not guide in that favorable direction. Many individuals drink to 18 cups of tea a day which is sweetened with molasses. Toward ring they prepare spruce beer from spruce tips, a popular beverage at has antiscorbutic value.

In the light of our present knowledge it is easy to see why these ople suffer from obstinate constipation, gastrointestinal disorders, ght blindness, scurvy, edema, and occasionally beriberi. The chil-en, although practically all undernourished, do not have rickets, nce their supply of fish provides vitamin D. Tuberculosis is very mmon. The women suffer to an unusual degree from amenorrhea. ervous instability increases suddenly in March or April, 4 or 5 onths after fresh food is lacking in the diet. Psychoses develop in rsons with a predisposition or under unusual strain, such as lacta-n or worry. The teeth of these people are badly diseased. Im-ovement or recovery from the effects of the winter diet regularly lows transition to the summer diet when more fresh foods become ailable.

The Diet in Newfoundland.—Appleton (l.c.) found a much gher incidence of beriberi in Newfoundland than across the raits of Belle Isle in Labrador. Xerophthalmia was frequently en-untered there. On the Newfoundland side, she found the people have no canned milk and no vegetables. She states that the New-undlanders are considered much more prosperous than people in brador, since they have many cows. Many of the Newfoundlanders some butter, which in the Labrador region is a rarity. But the cows not milked later than October to December of each winter. The y had been cut late that year (1921) after the grass had become dry, d many of the cows perished before the end of winter. Those which vived were in such poor condition that they did not recover until middle of summer. Cows were fed hay only and were never given t. Only with respect to the seasonal availability of milk is the food pply of the people of Newfoundland different from that in Labra-r. It is not uncommon, as pointed out by Aykroyd ('33), for a fisher-n who has subsisted for six months in winter largely on tea and ite bread, to develop beriberi which incapacitates him for fishing ring the next season. This places him at a still greater disadvantage he purchase of food for his family, and the next winter's supply will tain less of the better class foods. Thus the chief cause of the fficient dietary of these people is their poor economic status.

The Diet of Italian People.—The Italian people may be taken representatives of the Mediterranean region. Mariotti ('19) has dis-sed the diet of the better class of Italians. Many kinds of cheeses

are used in their cookery, and extreme care is taken to develop sp cial flavors in foods; for example, strawberries are never eaten wi cream, they are not washed in water but in wine, and they are serv with claret or lemon juice and sugar. Peaches are soaked in wh wine at the table. Sliced ham is served with fresh figs to bring o its flavor.

The peasantry live largely on cornmeal made into a mush or in a flat cake without yeast. The Tuscan and Umbrian peasantry a said to live almost exclusively upon a vegetable soup. Beet root, ca bage, broccoli, or potatoes are cut into small pieces and boiled water to which is added a small slice of pork chopped very fine wi an onion and some parsley. Tomato paste is added to this mixtur When served it has grated cheese sprinkled over it. It is said that on at Christmas and during the haying season do the peasantry drin wine or eat meat. No discussion of Italian food would be comple without mention of macaroni. Macaroni is only one kind of what called "pasta." There are many varieties of this foodstuff, of whic the more familiar examples are spaghetti and vermicelli.

Davis and Wood ('21) also reported on the dietary of the Italian They state that wheat, corn, and other cereals, vegetables, fruit chickens, pigs, and the milk of the goat constitute the food of th farmers. Fruits and vegetables are produced in great quantities i Italy and even the poorest people have them in abundance. Childre drink goat's milk, since every peasant has his goat, or else milk i obtained from his neighbor. The people consume little meat, and tha which is used is made into stews with macaroni and tomato sauc Eggs are used more abundantly than meat. Desserts of the Italian ar frequently fruits. The Italians make their own cheese from goat' milk. They are very fond of green vegetables, and in the large Ameri can cities Italians select in the markets a larger proportion of thes when they can afford them than do other nationalities. They fin milk and green vegetables relatively expensive and they substitut coffee for milk. Since they have been accustomed to liberal amount of Italian cheese, they buy this cheese at a dollar and a quarter pound rather than American cheese at fifty cents, or less, a pound. A taste for sweets is soon acquired in this country, and they also com to use the refined cereal products, since they are one of the cheape foods. Gillett ('22) states, "No milk and too much coffee and cand are the greatest factors of the nutritional problem of the malnourishe Italian children in New York City." Mudge ('23) has also describe her studies on the Italian dietary.

Sherman and Gillett found in a study of 92 families in Ne York City in 1917 that 48.9 per cent of the families were receivin less than the standard allowance of 1.44 gm. of phosphorus per ma

r day; 53.2 per cent were receiving less than 0.68 gm. of calcium and 3 per cent were receiving less than 15 mg. of iron. The study in e winter of 1918 showed the following increased deficiency:

	1918	*1917*
Families receiving less than the standard allowance of phosphorus	61.8	48.9
Families receiving less than the standard allowance of calcium	57.3	53.2
Families receiving less than the standard allowance of iron	51.2	41.3

Phillips and Howell ('20) in commenting on these studies, say at doubtless this deficiency in mineral content was one of the uses for the undersize and lack of vigor noted among the children the families studied. Among the Italian children there were many ses of rickets,—in fact, one would seldom see a child between 1 and years of age who was not extremely bow-legged. Sherman and llett found that in many homes no milk was bought because it was nsidered too expensive, but at the same time a small piece of lian cheese was purchased at a dollar and a quarter a pound, or ore.

Dietary Study in Poland.—The Polish diet may be regarded as presentative of the diet of many Slavic peoples. Morzkowska and cLaughlin ('28) have reported studies made in the fall of 1926 in land on Polish food habits. A Pole is more likely to begin the y with tea than with coffee. The staff of life among the peasants rye bread. This is a coarse black bread depending upon wild yeasts r fermentation. In the cities a finer flour and cultivated yeasts are ed. Baking powder is almost unknown. Several varieties of cereals ay a large part in the diet, but not in the form of prepared break- st foods. Barley, buckwheat, millet or wheat grists are made into ick porridges and sour soups, or are baked in the oven with salt rk and served with meats or milk.

A Pole eats about one-half as much meat as an American, but it ust be remembered that peasants make up about 40 per cent of the pulation, and that they eat meat, usually pork sausage, only on spe- l occasions, such as weddings or holidays. In the country, game, pecially partridges and wild hares, and smoked meats jerked over omatic fumes, are favorite delicacies; in the cities, brain, liver, tripe, ngs, and tongue are preferred to other meats.

Potatoes are eaten extensively. They form part of almost every eal. Cabbage, especially as sauerkraut, is popular. Beets are used ostly for making soups. Barscz, made from fermented beets with e juice of fresh beets added to give a red color, is a favorite soup. getables are cooked for a long time and are usually served in thick

sauce. Fresh fruits, though less abundant, are the same as i United States. Milk and eggs are used freely in the country, b milk supply for the cities is not well organized. Salt pork, mor butter and lard, is used for fat.

The Effect of Modern Transportation on Food Supplies Dietary Habits.—With the development of the steam engine, in the 19th century, transportation of foods over long distanc came more and more common. This is what brought about the mous development of the sugar cane industry in the West I and elsewhere, and the distribution of highly milled wheat wherever a market was available. In the not very distant pa peoples had a generous supply of sugar; and honey and sweet were the only sources of sugars. Today we witness the spectac nations such as Great Britain, the United States, and many others, suming 100 pounds and upwards of sugar per capita per year. S provides no proteins, vitamins or mineral elements. It tends to cr out of the diet foods which are better constituted for the mainten of health.

Most of the wheat growing regions of the world are far f the more densely populated areas. This is one of the important rea for the milling of refined cereal flours. From time immemorial ple sifted the flour ground between stones and made a somev refined product. But before 1879, when the roller mill process flour making was invented, milling was done in the same ma as in olden times—between the upper and the nether millsto This was a grinding process, and the different parts of the g could not be separated very well after grinding. The roller pro does not grind, but crushes the grain by pressure. When the w kernels are dried to the right degree, and the bran layer then allo to take up moisture, the latter toughens so it does not break up can be removed easily by sifting, as can also the germ which is disintegrated in the process. We have had thoroughly refined w flour only about 60 years. The principal incentive for extreme re ing of flour is to improve its keeping qualities so it can be ship anywhere in any climate, and stored without deterioration. On the most important factors in lowering the quality of the die many millions of Western peoples is the excessive use of refined f and other cereals, and sugar.

There is no valid objection to the use of these foods in cer quantities, provided they are supplemented with sufficient amo of the protective foods, namely, milk, eggs, leafy vegetables, and m The trouble is that this is not done. Here lies the principal prob of dietary reform in Europe, America, and other parts of the w where economic conditions will permit such changes.

REFERENCES

FFER (1921). "Studies in the Paleopathology of Egypt." U. of Chicago Press, Chicago.
INBECKER (1928). J. Biol. Chem., *80*, 461; ib. (1931), *93*, 327; ib. (1932), *99*, 279.
SINOWITCH and SMITH (1936). J. Nutr., *12*, 337.
ITH (1919). "The World's Food Resources." Henry Holt and Co., New York.
NG (1920). J. Home Econ., *12*, 289.
(1927). Chinese Social and Political Sci. Rev., *11*, No. 1.
ergovernmental Conference of Far-Eastern Countries on Rural Hygiene (1937). Health Section of the League of Nations, Official No. C.H. 1234.
NDEN BERGH (1921). "On the Trail of the Pigmies." The James A. McCann Co., New York.
NTINGTON (1927). "The Human Habitat." D. Van Nostrand Co., New York.
LIN (1861). "The Breath of Life." Trübner & Co., London.
OLIČKA (1908). "Physiological and Medical Observations Among the Indians of the Southwestern United States and Northern Mexico." Bureau of American Ethnology, Bull. No. 34, Washington, D. C.
ENTO (1928). "Causes of the Depopulation of the Western Islands of the Territory of New Guinea." Dept. of Public Health, Territory of New Guinea.
RSHALL (1926). Brit. Med. J., *ii*, 399.
Y (1928). "The Food of Japan." League of Nations Health Organization. C.H. 681.
ZAKI (1921). Annals Am. Acad. Political and Social Sci., *93*, 88.
LPH (1925a). J. Home Econ., *17*, 1; id. (1925b), China Med. J., *39*, 941.
K (1937). "Land Utilization in China." University of Chicago Press, Chicago.
VKS (1931-32). J. Am. Dietet. Assoc., *7*, 203, 375.
LETON (1921). J. Am. Public Health Assoc., *11*, 617.
ROYD (1933). "Vitamins and Other Dietary Essentials." William Heinemann Ltd., London.
IOTTI (1919). J. Home Econ., *11*, 337.
S and WOOD (1921). J. Home Econ., *13*, 19, 67.
ETT (1922). J. Home Econ., *14*, 14, 258.
GE (1923). J. Home Econ., *15*, 181.
LIPS and HOWELL (1920). J. Home Econ., *12*, 396.
ZKOWSKA and MCLAUGHLIN (1928). J. Am. Dietet. Assoc., *4*, 142.

CHAPTER

XXVII

Diet in Relation to the Teeth

DENTAL CARIES is the most widespread of human diseases. cayed teeth not only cause pain and the early loss of teeth w dental attention is not available, but are a menace to health. carious cavity is properly filled before the pulp cavity is invade tooth may be saved and be useful for years. If the pulp cavity been opened by decay it is necessary to devitalize the tooth and the root canal as an alternative to its extraction. This practice common years ago, but has fallen into abeyance because it was served that a tooth with a filled root canal generally developed abscess at the root end. Bacteria or their poisons found their way the blood stream from such abscesses and set up secondary infecti in such sites as the heart valves, joints, and kidney. In the ag gate, infected teeth have been and are a source of an immense amo of ill health, discomfort, and expense as well as early loss of te Any considerable improvement in the health of the teeth would, th fore, prove to be a great contribution to public health. This sub has attracted much attention in recent years and many facts of im tance have been established which have a bearing on the contro dental disease. As these facts are considered, it will become appa that there are several schools of thought which are in conflict a the cause of dental disease and the measures which should be ta to combat it.

Bunting ('36) points out that "The disease (caries of the tee is most unique. There is no other pathologic process which e remotely resembles it. Dental caries is not comparable with ca of the bone. It is not a true necrotic process, nor is it attended inflammatory reactions in the affected tissues. It is characterized the formation of progressive lesions in the teeth, simple decalci

by acids formed locally as a result of fermentation of carbo-
ates by certain aciduric types of bacteria. The process is dependent
ne infestation of the mouth by specific types of bacteria, capable
roducing acids by fermentation of residual carbohydrate food
rials in the mouth, and capable of living in their own acid
ucts." Bunting agrees with the fundamental concept of Miller
) that caries of the teeth always begins from the surface of the
and advances inward, a view now universally held. He goes
nd Miller in showing that not the number alone, but the kind
icroorganisms, is the determining factor. He holds that *Lacto-
lus acidophilus* is the specific agent which causes decay of the
because it is capable of producing a high concentration of lactic
by fermentation of sugar and can live and thrive in a strong
medium.

I. Mellanby ('29-'34), on the other hand, supports the thesis, on
basis of the most extensive and critical study of tooth develop-
t and susceptibility to dental caries which has been made, that
structure, together with those nutritional factors which play
e in calcification, are the most significant causes which operate
termine whether an individual will be caries-susceptible or caries-
une. Her conception of the etiology of dental caries includes,
fore, consideration of the importance of irregularities in the
nel surface (pits and fissures), the physiological state of the tissues
e pulp cavity, which nourish the living tooth, and the character
ne saliva which bathes their surfaces, as well as the degree of
ction of body chemistry as respects many factors, especially those
ucive to normal calcification processes. It would seem that her
s provide some explanation for the fact that *L. acidophilus* attacks
teeth of some individuals and leaves intact those of other
iduals.

Hyatt ('33) has approached the problem essentially from the point
ew of the dentist whose purpose is to prevent as far as possible
development of caries of the teeth. He points out that decay
ys begins in pits or fissures in the enamel. These are potential
traps and areas of potential stagnation which determine the
ce liability of the tooth to decay. Into these receptacles food
is is lodged, and fermentative organisms decompose the carbo-
ate moiety. At the bottom of such pits or fissures the nutrient
rial remains long undisturbed, and each affords a harbor, where
accumulates and remains in contact with the tooth substance
h it gradually dissolves, forming a cavity. As the food debris in
pits becomes liquified, a new deposit takes its place and so
process of etching is more or less continuous. Hyatt proposed,
fore, that the most effective method of preventing tooth decay

is for the dentist to search with unusual care for pits and fiss and when these are found, to drill a small hole in the tooth, enlar the enamel defect sufficiently to permit the insertion of a filling. surface is then polished, and the potential food trap is thereby ob ated. An extensive clinical experience by Hyatt and others w seem to have proven the efficacy of this procedure, which m involves finding the site at which decay will eventually occur, mechanically repairing the anatomical defect, thus preventing d

That the problem is not so simple as it would seem to be is sh by the study carried out by Palmer and his associates (Franzen to determine whether it is possible to prescribe a definite manne finding those occlusal surfaces of first molars which are liabl caries and those which are not. In this study of the first molar 1636 eleven-year-old boys and girls, they state that every effort expended to make the best use of training, supervision, and consu tion with dentists in making the observations. Nevertheless, they w unable to distinguish first molars which were liable to caries, f others not liable to decay. First molars which appeared to t observers to have no enamel defects were more liable to decay t were those which could be described by them in terms of pits fissures. First molars which had escaped caries at the age of 12 w found to have as much enamel defect as non-carious first mo in the mouths of six-year-old children. These experimenters c cluded that it is not possible to plan an effective program for ca prevention on the basis of detecting by examination the tooth wh is liable to decay. They believe that tooth decay is periodic and t it is a function of the individual organism and not of the tooth.

Hanke ('33) presents an imposing series of observations wh substantiate his view that tooth decay is, in general, due to an ins ficient supply of ascorbic acid (and possibly other substances provi by citrus fruit juices). He reports an arrest of about 50 per cent dental caries in children by the administration of a pint of ora or lemon juice daily in addition to an otherwise nearly suffici diet. The frequently observed gingivitis in children is reported clear up under this regimen.

A consideration of the course of development of the teeth a the influence of certain dietary factors upon tooth structure is nec sary in order to visualize the biological processes involved in etiology of dental caries, and to appraise the experimental a philosophic observations presented by the several schools of thou on the subject under review.

Tooth Development.—For our present purpose it may suffice state that the tooth consists of a crown of enamel overlying a struct of dentin to the neck of the tooth. The root consists mainly of dent

here is within the tooth a pulp cavity which contains the nerves, ood vessels, lymph vessels, and connective tissue.

Rosebury, Hastings and Morse ('31) arrived at a formula for amel represented by $CaCO_3n(Ca_3(PO_4)_2)$, where n has a value not less than 2 or more than 3. There are other constituents, such magnesium and fluorine which are in much smaller quantities an calcium and phosphorus. Qualitatively dentin has a similar composition but a different histological structure and it is not as hard enamel. The deposition of minerals, therefore, requires that the te of the body in general be suitable for calcification processes. here are specialized cells for the formation of the enamel and dentin, z., the ameloblasts and the odontoblasts. With few exceptions all the th begin to calcify from 4 centers, which eventually coalesce and come a tooth. It is frequently observed that these lobes where they alesce do not join in a perfect manner, thus leaving the tooth with

Diagrammatic illustration of structural flaws in the occlusal surfaces of molars and uspids: 1, enamel has no fissure; 2, normal fissure, can be readily cleansed; 3, fissure so narrow that food particles may lodge and evade removal; and 4, fissure forms a ottle-neck" in which debris will ferment undisturbed by ordinary cleansing. a, mel; b, b^1, dentin; and c, pulp cavity.

uctural flaws. This is illustrated by the accompanying figure which dicates how food particles may be lodged in fissures and evade noval by ordinary means.

Enamel Formation.—The enamel forming cells (ameloblasts) are rived from the same embryonic tissue (ectoderm) from which m epithelium has its origin, and although they are specialized for cification they are, like epithelium in general, extremely sensitive deficiency of vitamin A. The ameloblasts form a cap-like structure the inner surface of which enamel rods are laid down in a mosaic six-sided prisms, each cell forming a prism. In perfect enamel se are laid together so precisely that, when finished, the surface smooth to molecular dimensions. The first part of the enamel sm to be formed is the innermost end. As the enamel prisms gthen the ameloblasts retreat. The enamel is, therefore, formed m within outward.

In deficiency of vitamin A the enamel forming cells become a normal in appearance and instead of forming an even plane curved surface they tend to buckle so that when the enamel

Photographs of human deciduous teeth illustrating various degrees of hypoplasia (Courtesy of M. Mellanby.)

finished the surface presents an uneven contour like a landscape o hills and valleys. These cells, when inadequately nourished as respect vitamin A, do not lay down enamel of the normal density, but rathe

incompletely calcified product with interstices. M. Mellanby has cribed these abnormal processes in detail, and has shown that mpletely calcified (hypoplastic) enamel permits dyes to permeate o considerable depths. One can visualize the permeation of such oplastic enamel by water or saliva, carrying into the enamel terial which might serve as food for bacteria, and in severely oplastic enamel, microorganisms may be capable of finding their y below the surface of the enamel.

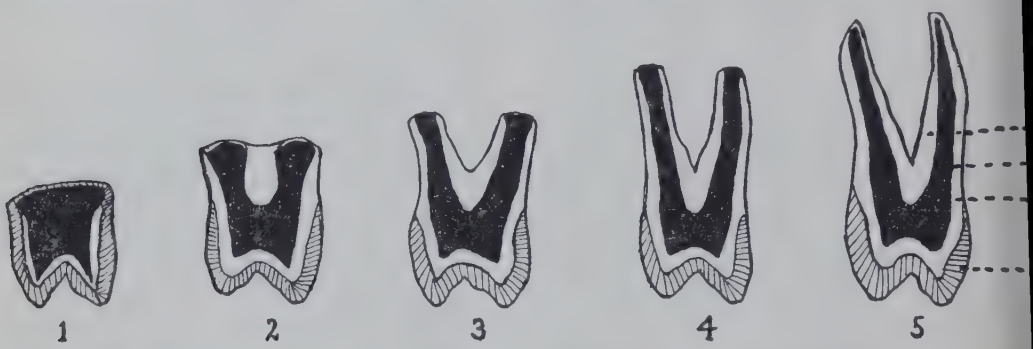
Photomicrograph of human deciduous teeth illustrating carious processes (c) in nel and dentin. (Courtesy of M. Mellanby.)

In case of localized buckling of the ameloblastic layer, or changes the individual cells so severe that each fails to form its enamel sm, localized areas on the completed tooth may exhibit fine tubes etrating the enamel to varying depths depending upon the stage development at which the injury to the ameloblasts occurred.

On the occlusal, or chewing surface of the teeth, the lines along ich the separate lobes of the developing tooth coalesce, as the tooth rs completion, there is considerable likelihood that the enamel as will not join perfectly when vitamin A is deficient in the diet. that case there will be a deep pit or groove in the enamel which y penetrate to the dentin. When food is forced into this pit or ove it tends to remain until bacterial action digests and liquifies it.

Such pits form nests of bacterial growth. Hypoplastic areas may oc anywhere on the enamel or within it. Such developmental defe as are here considered are well illustrated in the accompany photographs supplied by the courtesy of M. Mellanby (l.c.).

Dentin Development.—Enamel develops from within outwa dentin develops from the outer surface inward. After the earli deposit of enamel is formed, the odontoblastic layer of cells which in contact with it begin to lay down dentin and retreat inward the process extends. Thus, in a young tooth the pulp cavity is lar and becomes smaller as more dentin is laid down. The upperm portions of dentin are first formed, and the tooth lengthens as grows, the root tip being the last part to be constructed. Th processes are illustrated in the accompanying figure.



Diagrammatic illustrations of structural relationships in development of the huma upper first molar: 1, at 5th year; 2, at 6th year or the approximate time of eruptio 3, at 7th year; 4, at the 8th year; and 5, at the 9th year when calcification of th external parts of the tooth have been completed. a, enamel; b, b^1, dentin; and c, pul cavity.

Dentin is derived from the mesoblastic tissue and is, therefor embryologically related to bone. The odontoblasts, which form dentin are extremely sensitive to deficiency of ascorbic acid. If the tissue become depleted in this substance while the teeth are in process o growth, defective dentin will result.

But since all calcification processes are dependent upon a norma concentration of calcium and phosphate ions in the blood, bot enamel and dentin will be hypoplastic when the supply of the vitami D is deficient, even though the health of the ameloblasts and odonto blasts may be normal. It will be seen, therefore, that the dietar factors which most directly influence tooth structure are vitamin A and D, ascorbic acid, and calcium and phosphorus. It should b emphasized, however, that just as severe illness of any kind cause interruption in the growth of hair and nails, producing weak spot in the former and grooves in the latter, so such illnesses will caus

rruption in the development of the teeth, resulting in hypoplastic
s in enamel and dentin irrespective of the character of the diet.
The tooth is a living structure possessing a blood and lymph
ply in the pulp cavity. It contains processes, the Tomes fibrils,
nding from the odontoblasts into the canals which permeate the
tin to the base of the enamel. These carry lymph throughout the
tin.

The circulation of lymph throughout the dentin by way of dentinal
ules and the presence in these canals of dentinal fibers would
n not to be without purpose. The most obvious purpose would
n to be nourishment of the dentin. That there is a circulation of
ph in the dentin is indicated by the fact that the organic material
more abundant in proportion to the mineral substance in youth
n in later life. A circulatory medium is necessary for the transport
the calcium, phosphate, carbonate, and other ions, which are
osited after the maturity of the tooth. The matrix of dentin is said
e structureless but it is derived from connective tissue.

Dentin is capable of reacting to stimuli from without by the
nation of secondary dentin, which seems to represent a sclerosis
n dense calcification of the matrix. Thus, after injury or irritation
e may be formed immediately beneath the peripheral lesion a
ified barrier which seals off an area of injured or dead tubules.
certain that this method of protecting the tooth against destruction
s not serve effectively in man to arrest invasion of the dentin by
ous processes. But when caries-producing agencies cease to act
ppears that there is some attempt by the dentin to increase the
sity of the mineral deposit in the region bordering the cavity.

It is of extraordinary interest that in hyperparathyroidism, which
lts in mobilization and excretion of calcium salts from the bones,
dentin of the teeth is not decalcified. The teeth may fall out
ng to resorption of alveolar bone which destroys the sockets but,
hown by Albright, Aub and Bauer ('34), and others, rarefaction
he jaw bones stands out strikingly against the well-calcified teeth
yperparathyroidism. Schour and associates ('34) produced experi-
tal hyperparathyroidism by injecting an extract of the gland,
found rarefaction of the alveolar bone but not of the teeth.
eated pregnancies and lactations do not decalcify the teeth as
do the bones, as pointed out by Schour ('38) who has reviewed
subject.

Enamel, on the other hand, appears to be too dense to admit a
of fluid through the extremely tenuous organic matrix which
ls the enamel prism. It affords an outer fortification which repre-
s nature's best effort to produce a protective covering. If there

are breeches in this fortification, or the walls are crumbling in pla the tooth is more vulnerable than when it is of good construction.

Enamel is susceptible to dissolution by chemical agencies such the presence of acids or other substances which are capable of solving calcium phosphates. The teeth are constantly bathed by sali a fluid containing about 99.42 per cent water, and 0.58 per cent solids. Of the latter about one-third represents inorganic constituen and the remainder organic substances, including mucin, enzym and debris. Saliva is charged with carbon dioxide, which accounts its tendency to froth, and is so well buffered that it is approximat neutral in reaction. Extremes of pH values reported are 5.75 to 7. Most samples fall within the range pH 6.35-6.85. It is capable promptly neutralizing acids taken into the mouth or formed th by fermentation of sugar, except when the acid is shielded from cont with saliva, as in the case of a pit or fissure plugged with food deb or under a mucinous plaque on the enamel surface. Saliva is somewhat variable product, but from the standpoint of its functi as a protective coating of the teeth the essential qualities would see to be its approximate neutrality, its capacity to neutralize acids, a its content of calcium and phosphate ions, which, according to t laws of solubility, should prevent it from exerting a solvent acti on the calcium phosphate complex constituting the enamel.

Variation in Incidence of Dental Caries.—Ruffer ('21) recor many observations on the teeth of prehistoric peoples of Europe a of Egypt which make it certain that from the earliest times cari of the teeth have afflicted mankind in various parts of the worl There have been peoples at various times and places, however, wh were relatively or entirely free from this affliction. Weld (1795) stat that American-born men and women of the 18th century had decay teeth at an early age. We refer to this observation because the co sumption of sugar at that time was very small and, as we shall poi out, sugar *per se* is regarded by some investigators as the mo important factor in the production of dental caries. Catlin (186 noted the extraordinary perfection of the teeth of Sioux Indians, bo in the living and in skulls of past generations. Hrdlička ('08) stat that at the time of his observations dental caries was not rare amo the Indians of the Southwest, though much less common than amo the white people of the region. Various observers are agreed that t primitive Eskimos were free from dental caries, as are those wh today subsist upon their primitive diet of meats, fish, and other foo principally of marine origin. Price ('34) and others have commente upon the rampant caries of the teeth of Eskimos and Northwe Indians who have changed their diet to one of refined cereals a sweets. Ottofy ('08) found 68 per cent of Igorot children to ha

rfect teeth, and the remaining ones to exhibit imperfections so ght that they would have escaped notice if casually inspected.

Marshall ('26) visited the Island of Tristan da Cunha and ex- ined the teeth of more than one-third of the 141 inhabitants, 69 whom were adults: 21 were between the ages 14 to 21; and 51 ere under 14 years of age. The results of his examination were bulated as follows:

	Age Period			
	3–14	15–20	21–44	45–90
umber examined	13	8	21	12
umber of teeth	290	235	662	337
umber of carious teeth	2	0	11	21
umber of carious mouths	1	0	5	8
umber of teeth missing	0	0	6	45
rcentage of carious teeth	0.68	0	1.6	6.2
rcentage of carious mouths	7.6	0	23.8	66.6
ronic general peridontitis	0	3	14	7

The food of the Islanders is entirely home-grown. Fish are plentiful ut are not eaten extensively. Hen's eggs are scarce, but molly- auk's eggs are eaten in large quantity. Milk is nearly always entiful, and potatoes are the main source of carbohydrate. Onions, umpkins, turnips, carrots, beet-root, and leeks are grown, but in nall quantities. Butter, jam, and marmalade are rarely eaten. No read is eaten since cereals cannot be grown. The outstanding short- ges are flour, sugar, jam, and tea and coffee.

The data of Steggerda and Hill ('35-36), as well as those of other vestigators in recent years, confirm the high incidence of caries in ertain populations. The table on page 610 by Steggerda and Hill was ompiled from data from the mouths of 1358 Dutch in a Dutch ommunity (Holland, Michigan), 944 Maya Indians, 1861 Navajo ndians, and 599 Jamaica colored persons.

That the very high incidence of caries in the Dutch group is robably typical of dental conditions in the white youth of America supported by the data of Brekhus ('31) who examined 10,445 udents at the University of Minnesota whose average age was 18. Ninety-seven and one-half per cent were found to have caries and here was an average of over 10 cavities, or fillings, per mouth. Al- hough the following table shows practically no differences between Maya and Navajo Indians, it is important to point out that the diet of he former contained a high percentage of carbohydrate and the cavities

FIVE-YEAR GROUPINGS OF INDICATED RACES, SHOWING PERCENTAGE OF POPULATION HAVING CARIES, PERCENTAGE OF TEETH AFFECTED, AND AVERAGE NUMBER OF CAVITIES PER MOUTH

AGE	DUTCH (WHITE)				MAYA (INDIAN)				NAVAJO (INDIAN)				JAMAICA (COLORED)			
	No. Examined	Percentage Having Caries	Percentage of Carious Teeth	Average Cavities per Person Having Cavities	No. Examined	Percentage Having Caries	Percentage of Carious Teeth	Average Cavities per Person Having Cavities	No. Examined	Percentage Having Caries	Percentage of Carious Teeth	Average Cavities per Person Having Cavities	No. Examined	Percentage Having Caries	Percentage of Carious Teeth	Average Cavities per Person Having Cavities
6–10	702	46.9	4.2	2.4	373	3.2	0.2	1.8	647	5.3	0.3	1.6	3	...	...	...
11–15	533	88.2	12.8	4.3	319	10.7	1.9	1.5	634	17.0	1.0	1.7	304	69.4	10.2	4.1
16–20	123	96.7	24.1	6.9	106	37.7	2.5	2.0	491	32.6	3.3	2.8	144	71.5	18.6	7.2
21–25	...	...	...	..	76	60.5	6.1	3.33	60	58.3	7.4	3.5	86	80.2	24.0	8.4
26–30	...	...	...	..	43	65.1	7.2	3.7	21	66.7	7.0	3.2	44	90.0	26.6	9.4
30–35	...	...	...	..	25	72.0	8.9	4.4	8	50.0	9.4	6.4	21	95.2	28.3	9.0

ed to appear on the teeth with smooth surfaces. The diet of the r contained much protein and little carbohydrate and the cavities almost entirely confined to teeth having pits and fissures.

From the observations recorded it seems clearly apparent that the plays an important role in the etiology of dental caries, but how? studies of Hyatt (l.c.) deserve first consideration. He points out of about 400,000 teeth of 12,753 persons between the ages 16 to ears, 52.44 per cent of all the carious cavities were found on the usal surfaces of the bicuspids and molars; 5.90 per cent were on buccal surface of the lower molars; 4.44 per cent were on the ual surfaces of the upper lateral incisors, while the remainder 7.22 per cent were distributed over all the other surfaces.

Hyatt, as already mentioned, incriminates the pits and fissures on occlusal surface as the pre-carious defect, since these are food s in which lactic acid is formed from carbohydrate food remaining isturbed there because of the food pack, thus affording time for mel and dentin decomposition and resulting in the formation of vity. His view is in accord with the fact that people tend to be es-free who subsist largely upon food of animal origin, and hence e scarcely any fermentable food capable of forming acid.

On this basis we should have to account for the relative absence dental caries among peoples who eat much cooked starch, which uld be fermentable if packed in a pit in the enamel by assuming their diet was such as to induce the formation of teeth which no pits or fissures in their enamel. On this point we have no a sufficiently exact to be of value. Hyatt points out that the pits fissures on the occlusal surface of teeth are frequently so small a bristle of the smallest diameter found in tooth brushes cannot er; hence the futility of relying upon cleaning the teeth by brushing, other mechanical means. There is, however, much evidence that ning the teeth does afford protection to some extent on those ts of the surface which can be reached by mechanical means.

Nutritional Investigations of the Cause of Dental Caries.—The arches of Mellanby, which are confirmed by the work of several ers, have conclusively demonstrated that the character of the teeth jaws, as regards their general development and their microscopic cture, can be greatly influenced by diet. If the diet of a puppy is nged from one poor in those dietary factors concerned with tooth nation to one rich in these substances, there is an immediate onse shown by improved calcification of the developing enamel dentin. In her experiments, improper feeding of the mother ing pregnancy had a deleterious influence on the tooth structure he pups. Obviously this is a matter of degree since Klein, Mc-lum, Buckley and Howe ('30) found that pregnant sows confined

to diets which, when fed to pigs after weaning, promptly i rickets, made sufficient sacrifice of their own nutrient rese insure essentially normal development of the teeth of the yo birth. The mother also made sufficient self-sacrifice during la to afford great protection to the suckling pigs. In both cas health may become impaired.

M. Mellanby also found that, notwithstanding hypoplasia, of the teeth developed in but 2 of 28 dogs, and in these there pre-carious cavities in the enamel. The presence in the food of microorganisms said to be responsible for dental caries in ma *Streptococcus mutans* and *L. acidophilus,* did not in her exper induce caries, whether the diet possessed high or low calcifying erties. Likewise, the addition of even large amounts of gluc fermentable sugar, did not induce caries.

Bunting and his associates, starting with full confidence i theory of Miller (l.c.) that acid decomposition of the teeth cause of caries, have carried out an extremely illuminating ser studies on the etiology of dental caries. Hubbell and Bunting examined the saliva of 125 children of varying caries-suscepti of ages 7 to 16 years. Some of the children received supplemen milk, viosterol, and tomato juice. The calcium and phosphorus varied widely from day to day in the same individual and no rel ship to the degree of activity of dental caries could be foun those children receiving the supplements there appeared to be a decrease in the incidence of dental caries but no accompanying ch in the salivary calcium or phosphorus could be found.

Hubbell ('33) made a more detailed study of 32 children, 9 years of age, 15 of whom were caries-free and 17 distinctly c susceptible. Both blood and saliva were analyzed chemically. results showed that the values for calcium, inorganic acid-so phosphorus, carbon dioxide capacity, and pH were the same in groups. In the saliva the values for total solids, ash, calcium, phorus, chloride, diastatic activity, and pH showed no cons difference in the two groups. A difference in the salivary acid ne izing power was noted in that the average carbon dioxide cap of the caries-free group was about 44 per cent higher than in caries-susceptible group, and the average titratable alkalinity w per cent higher in the caries-free children. There was a wide of variation in each group, however, and the values were not ficiently constant for each individual to be of significance.

Koehne, Bunting and Morrell ('34) found that the carbon di capacity and the alkalinity of the saliva of children in whom had been arrested by dietary control, were not consistently h than during the earlier period preceding dietary control. The alka

the saliva could not be raised by increasing the basic constituents the diet.

Hubbell and Koehne ('34) increased the caloric intake of children to 18 per cent by added sugar, and found a tendency to increased rogen and phosphorus retention, but a slightly decreased retention calcium. White and Bunting ('35) observed no significant differ- ces in the pH and ammonia content of saliva in caries-susceptible d caries-immune children. Bunting and his coworkers conclude, erefore, that the diet does not affect the composition of saliva in y known way which may influence susceptibility to caries of the th.

On account of the wide variations in morphology and cultural aracteristics, some investigators have questioned the identity of the ganisms of dental caries with the *L. acidophilus* group commonly esent in the intestine. Hadley, Bunting and Delves ('30) studied ese variants obtained under laboratory conditions from oral and testinal strains of *L. acidophilus* and found that the cultural dif- rences between oral and intestinal strains also exist between in- stinal strains as a group, and that there is no justification for parating these into distinct groups. They described three forms of lonies, viz., smooth, intermediate, and rough forms of oral strains d produced many dissociation variations between them. The organ- ms associated with dental caries vary widely in type and char- teristics, but belong to the general group of *L. acidophilus,* and e related to those occurring in the intestine. Hadley and Bunting 32), by means of a rough quantitative method, found an average of ,000 *L. acidophilus* organisms per cc. in the saliva of 14 caries- sceptible children, as against an average of 600 in 10 caries-immune ildren; many counts being negative and in 81 per cent of the unts the number was less than 100 per cc.

Jay, Crowley and Bunting ('32) prepared a vaccine from 40 oral rains of *L. acidophilus* isolated from cases of dental caries. When is was introduced intradermally into caries-susceptible individuals, finite cutaneous reactions were produced in nearly all cases. Similar oculation of caries-free individuals produced no reaction in the ajority of cases. This finding indicates the existence of some form sensitization to the organism. In two children who were caries- sceptible and gave positive reactions to the vaccine, the *L. acid- hilus* agglutinins were increased in the blood and the skin reactions the vaccine became negative. In a later study Jay, Crowley, Hadley d Bunting ('33) observed positive skin reactions in 90 per cent of caries-susceptible persons. In 40 caries-free individuals similarly cated, a positive reaction was observed in 12, or 30 per cent. The cal reactions to the vaccine were so severe that further study of this

problem was discontinued until means become available to prev the unfavorable sequellae.

These important researches appear to have established that org isms of the *L. acidophilus* group are present and growing in lar numbers in practically all mouths in which caries is active, and a either absent or sporadically present in small numbers in practica all caries-free mouths. The organism is similar to the *L. acidophil* commonly found in the intestine. The researches have also broug to light the existence of some form of immunologic principle whi influences the growth of oral *L. acidophilus*. Bunting and his associat have found it impossible to implant the organism in many caries-fr mouths, and in such individuals the presence in the blood serum an agglutinin for the organism can usually be demonstrated. The have been able in a few individuals to raise the agglutinin titre again the organism, thus suggesting the possibility of development of vaccine therapy against dental caries.

In the nutritional studies of Bunting's group the conclusion reached that in a large percentage of cases the feeding of an adequat diet, in which the content of sugar is reduced to a low level, reduce the *L. acidophilus* count of the saliva, and decreases or arrests, denta caries. Furthermore, they found that the addition of much sugar t an otherwise normal diet will, as a rule, greatly increase the growt of *L. acidophilus* in the mouth and will stimulate the activity o dental caries.

A nutritional study was made by Koehne and Bunting ('34) o a group of 169 orphanage children of 7 to 16 years of age. Thes children had been observed over four and a half years during whic time repeated bacteriological examinations were made and the insti tutional diet was checked at intervals. They had a remarkably lo incidence of active dental caries. Seventy to 80 per cent of them wer known to have had no new caries over a period of several years although many of the children had open cavities which had bee formed in earlier years, or previous to their coming to the institution The salivary *L. acidophilus* content was also remarkably low, th great majority of cultures being negative. The correlation of thes counts to the degree of caries activity in the whole group was 8 per cent.

An appraisal of the diet showed that children over 8 years o age received milk and oleomargarine routinely for breakfast, an occasionally for supper. No butter was provided. A high proportio of bread and other cereal foods was used regularly. Many vegetable and some fruits were raised on the institution farm. Apples, carrots tomatoes, onions, potatoes, cabbage, beets, and turnips were abundan at all times. Celery, spinach, peas, string beans, corn, berries, etc.

ere given to the children in season. Carrots, onions, cabbage, turnips, nd tomatoes were frequently served raw. Oranges were provided ly during the Christmas holidays, except when sent to individual ildren at other times by relatives. Canned berries, peaches, and ples, together with dried prunes and peaches were purchased. Fresh nanas were occasionally provided. Eggs were available to the ildren during the spring when they were produced on the farm in nsiderable numbers. The only desserts given regularly were fruit latin for Sunday dinner and cookies served once a week. Raw ple was used in place of dessert for dinner and supper at practically times. Fresh and smoked meats were included in the menus. Meat as served almost every day but the portions were quite small. andy sent to the children was not given to them. Relatives and ends were urged to limit food gifts to fresh fruit. No sugar was aced on the table and but a minimum was used to sweeten foods. Most of the children were below the average (Woodbury tables) both height and weight, and their caloric intake was estimated to lower than the minimum requirement. Their vitamin D supply as derived essentially from eggs and from sunlight.

In order to test the effect of sugar in the diet 51 of these children re each given approximately three pounds of candy each week er a period of five months and the activity of the oral *L. acidophilus* d dental caries was observed. Previous to this experiment all of se children had been restricted to the regular institutional diet, ich was uniform and low in sugar. During the previous year only showed any evidence of active dental caries. Throughout the 5 nths in which the candy was provided, and was eaten in addition the regular diet, the oral acidophilus counts were markedly in-ased in 80 per cent of the children, and at the completion of the iod 44 per cent showed evidence of active dental caries. Three nths after discontinuance of the sugar feeding and return to the sugar ration, a subsequent examination showed that in practically cases the acidophilus counts had dropped to their former levels d that there was no further extension of caries in any case.

Koehne and Bunting ('34, l.c.) state that observations on a group public school children living in their homes in Ann Arbor, chigan, seemed to indicate that irregularities in meals and in type food eaten day by day, associated in most cases with liberal use artificially sweetened food, encouraged cavity formation. They e that they have observed several orphanages in which it is the ctice to accept all gifts of confectionery which are frequently made well-meaning business firms and to dispense such food liberally to children. Among such children the incidence of dental caries responds to that found in public school children.

Koehne and Bunting suggest that the secret of controlling cari activity through diet may, for the majority of persons, lie more what is withheld from the diet than on the nutritional adequacy what is permitted. They make an important comment relative the significance of acquiring a knowledge of the previous history caries incidence among children before beginning observations on t effect of any prophylactic measures designed to test their efficien in the control of dental caries. Only 32 of the 169 children in the orphanage study were free from caries when examined in Octob 1931. The remaining 137 had teeth showing varying amounts decay, and open cavities occurred in many instances. If they ha begun to give viosterol or other treatment at that time, without a previous knowledge of caries incidence among the children, a had based their conclusions on clinical findings in October, 193 they would have been able to present striking evidence for the ben ficial effect of any treatment which might have been applied.

In the light of Bunting's conclusions the consumption of sugar several countries is of interest. The following figures were compile by the International Institute of Agriculture ('36):

PER HEAD CONSUMPTION OF SUGAR PER YEAR (PERIOD 1930–1934)

	Pounds		*Pound*
Austria	61	Norway	70
Belgium	62	Poland	23
Bulgaria	10	Roumania	12
Czechoslovakia	56	Sweden	95
Denmark	120	Switzerland	98
Finland	51	United Kingdom	110
France	57	Canada	95
Germany	52	United States	103
Italy	18	Australia	107
Netherlands	68		

Tooth Decalcification in Relation to Candy "Sucking."—Mille and Neuwirth ('35) are in agreement with Hyatt on the point tha dental caries, or the pre-carious pit or fissure, when properly treate mechanically, can be arrested, often never to recur at the original sit Decalcification of the enamel, they point out, presents an entirel different situation. They have studied many patients whose teet were undergoing rapid dissolution, and have discovered that this traceable to exposure of the enamel to frequent baths of strong suga solution in the saliva. This occurs when the subject is addicted t sucking or biting hard candies, lozenges, flavored wafers, or coug drops. Any type of candy eating which involves slow dissolvin would accomplish this end, of subjecting the teeth for considerab periods to a strong sugar solution. Persons who smoke large numbe of cigarettes were frequently noted to form the habit of holding

ppermint, or other candy, between the teeth between smokes or uring smoking. Such patients were found to develop large numbers cavities in all the teeth. Such teeth become sensitive and the cavities come carious. It is pointed out that this habit spoils the appetite, nd that the eating habits become such as to bring on malnutrition om imbalanced diet.

Discontinuing the use of candy in all forms, reducing the amount smoking, adoption of a daily tooth brush drill, as well as a good et, relieved the patients of the progressive decalcification. The teeth sumed increased luster and sensitiveness disappeared.

It is not certain at present whether a strong sugar solution will ecalcify enamel, or whether acid formation through the agency of icroorganisms is a necessary factor in this process. It seems very obable that excessive candy eating would result in the flowering t of a profuse flora of *L. acidophilus* organisms in the mouth with nsequent acid formation. In this connection it is interesting to note at hard candies, in addition to flavoring, usually contain a consider- le amount of citric acid. Dissolution of enamel may be referable this acid.

The Natural Protective Coating of Enamel.—All dentists seem agree that in many mouths the teeth fail to decay although they e hypoplastic, the enamel presenting the rough, stained appearance ustrated by photographs in M. Mellanby's reports on experimental udies of dental caries. Such teeth are covered by a glary film that ves the mouth a filthy appearance and yet the teeth fail to decay. his fact has been brought forward as an argument against the view at the character of the enamel has anything to do with susceptibility dental caries. Of interest in this connection is the work of several vestigators with regard to the "protective film" found on many eth. Chase ('26) gives a thorough review of the theories concerning e nature of this film. Bibby and Van Huysen ('33) have discussed nature. Several investigators are agreed that when a tooth is treated th acids, a membrane-like pigmented structure can be separated om the surface of the crown. This membrane is shown to consist a bacterial mat composed of gram-positive thread forms, together th other bacterial types. The amount of mucin in this film is gligible. As a result of the formation of this superficial film the face becomes resistant to the destructive action of acids and bacteria, d the enamel is protected from decalcification and hence against ntal caries.

This view is interesting since it has been reported by Enright d Friesell ('33) that simple lactate or citrate buffer solutions will solve enamel slowly. Saliva is generally said to be saturated with cium and phosphate ions, and if this is so it could not dissolve

enamel. We do not know that such saturation always exists. Enrig and Friesell state that lactate or citrate buffer solutions saturated wi tricalcium phosphate dissolve enamel only at hydrogen ion concent tions more acid than pH 5.0. They found that the only microorganis which is ordinarily found in the food debris on the teeth which c tolerate and grow in an environment more acid than pH 5.0 is *acidophilus*. Their conclusions concerning the role of this organis in tooth decay are in harmony with those of Bunting. Apparently t protective covering on normal enamel is due to the growth of son calcium-fixing organism, probably *Leptothrix,* which has been show to form calcareous concrements and are probably the cause of t deposit of tartar on the teeth.

These findings are of interest in connection with certain observ tions of Simonton and Jones ('27) who described a destructive ty of deposit on teeth. In experimental studies on dogs fed deficie diets, stained areas which were white, cream, gray, yellow, gree brown, or black, occurred as spots or broad masses, sometimes affec ing the incisal tips of the teeth, or as bands around the teeth occurrin on various areas of the crown. Disintegration of the enamel occu coextensive with the areas of stain. In some cases the affected are were soft, in others, dry and chalky. The lighter colored stains seeme to be associated with the more active processes. On scraping thes areas it was found that the enamel was softened so that it could b easily removed, thus exposing the dentin. In other cases, after surface layer of soft enamel was scraped off, hard, vitreous enam was encountered. In some the entire crown was soft and could easil be cut away leaving merely stumps of dentin. They found that suc areas might be microscopic as well as macroscopic in size. The co dition was associated with rickets-like symptoms which occurred i young dogs, or in dogs restricted to a diet containing an excess c basic ions. Simonton and Jones never observed this condition i unerupted teeth and concluded that it does not represent a develo mental fault. They showed clearly that the softening starts fro without and advances into the enamel. This condition was attribute to an oral bacterio-chemical complex related to some systemic co dition associated with the ricketic state. These investigators pointe out that the conditions in the teeth in rickets are the opposite c those in scurvy. In rickets there is progressive failure of calcificatio first incomplete calcification, then the formation of organic matri alone, and finally, cessation of development. In scurvy it is the forma tion of the organic matrix which is first affected. In rickets the toot may present a large pulp and shell-like walls; in scurvy the pul tends to become solidified by the formation of pulp bone. Since thi condition, leading to disintegration of the enamel, is in no wa

ciated with caries of the teeth, and develops after the enamel is ned, it seems probable that it is due to some peculiar oral flora ch is enabled to flower out when the systemic condition of the nals becomes altered as respects certain inorganic elements, notably ium and phosphorus, and an excess alkalinity of the diet. It would ear that the stained areas described by Simonton and Jones repre- microbial growth of a kind or kinds not yet identified. In this nce we see the formation of a tooth coating which is destructive er than protective.

Arrest of Dental Caries by a Diabetic Diet.—Boyd and Drain '28) made the important observation, based upon repeated routine nination of patients in the dental division of their pediatric clinic, many instances of definitely arrested caries of the teeth were untered. Large cavities, which ordinarily would have an area of ned dentin surrounding the zone of destruction, were found ad to be walled off with very dense dentin. In these mouths, hich there was evidence of rapid and extensive involvement of y teeth, subsequent examinations showed that further invasion been checked, and open cavities did not show signs of progress ths after they had been first observed. The occurrence of salivary li on these teeth was almost universal, and it recurred rapidly its thorough removal. Salivary calculus is not uncommon in the t, but is unusual in the child. Otherwise these children did not anything noteworthy from the dental standpoint. Shedding and tion were about normal in most cases. The degree of oral hygiene d greatly as in any unselected group of similar size. Some teeth ing unquestionable arrest of caries were found in the most poorly mouths.

Upon correlation of these observations with the medical histories ne children it was found that without exception the ones with ted caries were diabetic patients who had been under careful agement for 6 months or more. That diabetes itself affords no ection against dental caries was indicated by the fact that a review e earlier examinations showed that the carious lesions were active e time of admission of the children to the hospital. Since arrest ries was not found in any other disease or condition, it appeared it must depend on some factor in dietetic management. Further established a parallelism between the establishment of diabetic ol and the quiescence of caries.

he diet for the control of diabetes differs from the usual concept n ideal diet for a normal child in that fat, rather than carbo- ate, is used as the chief source of energy, the fatty acid: dextrose being 1.5:1. All the children were maintained on the same ratio rotein:carbohydrate:fat, viz., 7:9:21. Adequate insulin dosages

kept the blood sugar concentration within normal limits, and g cosuria was infrequent. Boyd and Drain point out that when diab is well under control the diabetic child may be regarded as essenti a normal individual. Insulin administration is regarded as having effect other than the maintenance of normal sugar metabolism. these premises are granted the only factor in the arrest of caries the character of the diet. The diet was adequate in all respects the normal development of the child. It provided an abundance dairy products, fruits, and vegetables, and was supplemented w cod liver oil. The provision of an adequate supply of mineral sa and vitamins was assured. They suggest that the preponderantly ba ash content of the diet was of significance. The management of th children differed from that of normal children in that the diabe child was not allowed to use his appetite as an index to his food nee his menus being determined by the choice of foods offered hi Their conclusion that these diabetic children ate regularly a diet mo in accordance with the needs of a normal child than the avera child receives, seems justified. They conclude that dental caries is t sequel to recent dietary inadequacies, such as may be associated wi the usual food intake of the average child.

Boyd, Drain and Nelson ('29) recommend the following bas formula for the diet of normal children in the control of dental carie

Daily Intake

One quart of milk
One serving of meat or fish
One orange or tomato
One additional fruit
One teaspoonful cod liver oil*
Six teaspoons butter
One portion (½ cup) leafy vegetabl (spinach, chard, greens, cabbage, et
One portion (½ cup) meaty green veg tables (peas, beans, carrots, etc.)

* Other standard sources of vitamin D would be satisfactory.

They describe a typical day's menu, which includes the bas formula components, as follows:

Breakfast	*Lunch*	*Dinner*
Orange juice	Cream of tomato soup	Beefsteak
Wheatena with milk	Crackers	Baked potato
Poached egg	Salmon salad	String beans
Whole wheat toast	Bran muffin	Spinach
Butter	Butter	Tomato and lettuce sala
Bacon	Apple	Whole wheat bread
Cocoa	Milk	Chocolate pudding
		Milk

The above menu for the day in the amounts used provided protein 92.1, fat 123.1, carbohydrate 194.8, calcium 1.337, phosphoru 1.573, and iron 0.0212 grams, respectively.

Boyd and Drain have postulated on the basis of their observations, plemented by extensive studies on normal children placed on pervised dietaries, that dental decay is dependent upon some dietary ficiencies, and that any diet which contains an abundance of all recognized essentials for normal nutrition should be adequate to rest dental decay. They noted that if the child actually eats the ods of the character and in the amounts prescribed, the bases of e cavities harden within two or three months through deposition secondary dentin, and no further progress is noted. Starches and eets, they emphasize, should not be permitted to displace the more luable fruits and vegetables.

Boyd, Zentmire and Drain ('33) made a bacteriological study of children kept under strict dietary control along the lines indicated, d concluded that the nature of the oral flora and its ability to oduce acid, cannot be correlated with incidence of dental caries. hey observed that *L. acidophilus,* and other organisms of equivaltly high acid-producing power, may exist in large numbers in ouths persistently free from active caries. Children receiving diets such a nature that caries becomes inactivated and remains so, show characteristic change in the morphological nature of the oral flora in acid-producing capacity of the organisms. The incidence of *acidophilus,* and of other organisms having similar acid-producing bility, appeared as great in frequency and numbers in the mouths children receiving continuously such protective diets as in the ouths of those whose diets were not supervised. They conclude, erefore, that no available facts demonstrate that bacteria can of emselves induce caries of the teeth of individuals whose diet is dequate.

Boyd, Drain and Stearns ('33) carried out extensive metabolic udies of children with dental caries. They concluded that the levels serum calcium and phosphorus, and the acid-base relationships are ot primarily of significance in the production of dental caries, and e same is true of the chief constituents of the saliva. They found definite and, they believe, a very significant relation to exist between dequacy of retention of calcium and phosphorus, and resistance to oth decay. Their data offer no evidence that moderate dietary fficiency of phosphorus is an outstanding factor in the incidence of ntal caries, or that degrees of acid-base imbalance induced by any dinary diet can be held responsible. They indicate that a close rrelation exists between metabolic efficiency of the organism as a hole and the resistance of the teeth to decay. According to their ews, resistance to decay of the teeth is dependent primarily upon ctors operating within the tooth.

McBeath ('32) made a study in three orphanages, employing the

FOOD ANALYSIS

Weight in Grams per "Man" per Day

INSTITUTION	MILK	MEAT	FISH	ANIMAL FAT	VEGETABLE FAT	CEREALS	GREEN VEGETABLES	ROOT VEGETABLES	LEGUMES		FRUITS	
									Fresh	Dried	Fresh	Dried
I	706	110	24	45	Very little	417	104	353	2	5	27	4
II	772	98	22	45	Very little	433	89	316	18	6	31	10
III . . .	640	130	20	48	Very little	467	86	384	23	4	30	11

Vitamin Content per "Man" per Day

INSTITUTION	VITAMIN D	VITAMIN A	VITAMIN C
	I. U.	*I. U.*	*I. U.*
I	410	1,290	1,480
II	450	1,280	1,480
III	380	1,340	1,460

Supplements added to these diets:

I Daily addition of 28 to 42 grams of "Golden syrup"
II Daily addition of 14 to 21 cc. of olive oil
III Daily addition of 14 to 21 cc. of cod liver oil

inary diet of the homes. For the control groups he used the same ts modified by supplements of eggs, vegetables, fruits, and cod liver The studies covered 6 to 7 months, and the incidence of new ities during the experimental period was compared. The experintal groups all showed a smaller increase of caries than the control ups. Analyses of the diets failed definitely to correlate the protive action with any one factor, though vitamin D was the most rkedly increased factor in all the protective diets. In a fourth study effect of adding ascorbic acid in the form of bananas, oranges, or sh tomato juice was observed. The results did not support the w that ascorbic acid is of primary significance in preventing dental ies.

M. Mellanby (l.c.) studied children in 3 institutions at Sheffield d Birmingham to determine the effect of a supplement of vitamin on the incidence of tooth decay. The diets are described in the le on page 622.

Space does not permit a detailed presentation of the extensive a secured and analyzed in these institutional studies. The original ort should be consulted. Her results show that the percentage of ies in these children was lowest when cod liver oil was added as supplement and was highest when a sugar addition, as syrup, laced cod liver oil. A supplement of olive oil instead of cod liver protected against caries to some extent, the incidence being lower n when syrup was given. Olive oil contains no known vitamin. would appear, therefore, that raising the fat content of the diet, art from its vitamin content, exerts a protective effect against dental ies. This is of interest in connection with the effects of the high-fat -carbohydrate diet employed by Boyd and Drain with diabetic ldren on which caries became arrested. Does this mean that protion against caries may roughly parallel the replacement of carbodrate by fat in the diet?

BOYD AND DRAIN DIET FOR DIABETIC CHILDREN

(Boyd and Nelson, '26)

ROTEIN	CARBO-HYDRATE	FAT	POTENTIAL		RATIO	CALORIES
			Glucose	Fatty Acid		
Grams	*Grams*	*Grams*				
.0	1.28	3.0	2.165	3.16	1: 1½	36.1
.0	9.0	21.0	15.16	22.12	1: 1½	253.0
.76	3.55	8.3	5.95	8.74	1: 1½	100.0

The foregoing table shows the relationship between the var factors of the diet when the protein, carbohydrate, and fat are in ratio of 7:9:21 respectively.

In the diet employed by Mellanby, the CHO:Fat ratios were

I. 1.0:0.201;
II. 1.0:0.18; and
III. 1.0:0.25

These represent a fat to carbohydrate ratio of about one-seve that used by Boyd and Drain in the treatment of diabetes.

In a more recent study McBeath and Zucker ('38) reported investigation of over 800 children, most of whom were under obse tion during 4 years, to determine the influence of a supplement vitamin D on the incidence of dental caries. Their data clearly sh that dental caries is seasonal, the highest incidence being in winter and early spring after the effects of the winter diet had beco manifest, and was lowest during the summer. Their study points the beneficial effects of vitamin D in preventing caries. The ministration of graded amounts of vitamin D in the form of vitar D milk resulted in graded caries prevention. Of the three levels gi (250, 400, and 800 I. U. daily) only the last named was adequate prevent an increase during the height of the caries season above t of the previous period.

Fortifying the diet with "protective foods" or simply increas the allowance of milk led to a moderate reduction in caries when appreciable vitamin D was added. The change was clear cut in autumn-winter period but not so definite in the winter-spring peri A reversal of "control" and "experimental" regimens during two s cessive years in over 100 cases showed that individual susceptibil to caries was negligible compared to the effect of nutritional facto These investigators also found that irradiation of children with ult violet light had a favorable effect in reducing the incidence of car which is in accord with the lower incidence during summer. appears from their data that the administration of vitamin D in form of vitamin D milk is the full equivalent of summer sunlig in reducing susceptibility to tooth decay. McBeath and Zucker's stu fully substantiates the findings of M. Mellanby.

Ascorbic Acid and Dental Caries.—Hanke ('31) discussed various theories concerning the etiology of dental caries and conclud that the prevention of this disease cannot be attributed to any d factor of the diet but a liberal ingestion of all the essentials. In extensive study he found that the provision of a pint or more da of orange juice to a diet otherwise nearly normal not only arrest caries but brought about marked improvement in the health of t gingival tissues. His theory of the effectiveness of a high intake

rbic acid in preventing dental caries, or in arresting it, is based n the findings of Höjer ('24), which have been set forth in detail Chapter XVII. These relate particularly to the impairment of the lth of the blood capillaries in ascorbic acid deficiency. The daily regimen prescribed to patients by the dentists associated with nke's study, and which is reported to have given excellent results ractice consists of the following:

8 ounces of milk for adults, 32 ounces for children.
1 egg a day, except for adolescent children and pregnant women, who should have two eggs a day.
One-fourth to one-half head of lettuce.
Half a pound of fruit a day besides the orange juice.
Four-fifths to one pound of vegetables.
Meat once a day.
One pint of orange juice plus the juice of one lemon.

Other than these requirements the subjects were left to eat what y liked.

Hanke attempted to correlate the incidence of dental caries with occurrence of some one type of acid-producing organism, but in he was unsuccessful. He found some relationship between the sistence of *L. acidophilus* and cavities in the teeth, but in his study organism was not always found, even in mouths highly susceptible dental caries, and it occurred rather frequently and consistently in mouths of children who had never had any dental caries, or in om no new carious lesions developed during a period of two years. Hanke presented the results of his entire studies in book form , l.c.). The illustrations afford impressive evidence of the effective- s of dietary treatment in these conditions but, for purposes of rpretation, it would have been of great value had he conducted eries of experiments in which boiled orange juice was employed, rule out the possible effects of factors in orange juice other than rbic acid. The data available at present seem to indicate that the ximum content of ascorbic acid in blood and tissues can be secured a daily intake of citrous fruits, or other raw vegetable foods, siderably below what he considered necessary. It has been pointed that the data of McBeath (l.c.) are not in agreement with those Hanke.

The Experimental Production of Dental Caries in Animals by t.—M. Mellanby (l.c.) failed with few exceptions to produce erimental caries in the teeth of dogs. Various diets were employed. ne of these contained large quantities of glucose and other sticky mentable foods. Others consisted of diets including either large or

small amounts of vitamins A and D. Administration of *Strepto mutans* or *L. acidophilus* also failed. In some cases she re enamel to assist lodgment of debris. The almost complete a of caries under such conditions made it obvious that the dog is tically immune to dental caries.

McCollum, Simmonds, Kinney and Grieves ('22) reported a of the lesions in the teeth of rats fed deficient diets varying pri in the amount of vitamin A, calcium, phosphorus, and protein. observed "caries-like" lesions in over 5 per cent of rats fed a di in calcium and vitamin A, and less than 2 per cent in those comparable diet rich in vitamin A, whether the calcium was or low, but none was found in their stock animals fed a good diet. At that time vitamin D was unknown and their diets deficient in this substance. Similar results were reporte Toverud ('36).

Howe ('27), working with monkeys, states that the most r occurring and pronounced caries in these animals occurred whe diet was largely composed of cereals and gelatin; that a deficien ascorbic acid played a part in the production of caries; that exc sugars and starches did not cause caries in 9 months; and monkeys fed milk, vegetables, and fruit had excellent teeth.

Hoppert, Webber and Canniff ('32) reported that rats fed containing coarsely ground cornmeal developed caries of the but when the cornmeal was sifted and only that portion wa which passed a 60-mesh sieve, no caries was produced. They sugg that caries was the result of impaction of the deep grooves, v are natural in rats' teeth, with coarse cereal particles. The plu cereal underwent dissolution and fermentation produced acid, v was held *in situ* by the plug, so the saliva could not neutrali This is the old story of acid dissolution of enamel and dentin. T is every reason to accept their explanation. The rat's molar particularly designed to make them susceptible to dental di under these conditions of feeding.

Klein and McCollum ('31) examined 750 rat skulls with purpose of determining the relation between the age of the a and the incidence of caries of the teeth. They concluded that fed a caries-producing diet until 60 days of age do not show c caries. On the same diet, at ages 81 and 165 days, caries was obs in 88 per cent of the animals. Rats fed the stock diet to 60 da age did not have carious teeth. Between 300 to 500 days of certain number exhibited caries. The most probable explanatio these facts would seem to be that in rats on a caries-producing the enamel is defective, whereas in those on a non-caries prod diet there is enamel in the deep grooves, which is thicker, more

nd free from fissures; hence periodical plugging of the grooves, with onsequent acid action, requires a longer period to etch away the namel.

The view has been held by several investigators that the content nd ratio of calcium and phosphorus in the diet, and more especially deficiency of phosphorus, may be the determining factor in the tiology of dental caries. Failure of salivary analyses to reveal sigificant differences in the phosphorus or calcium content of the saliva eaves this theory with little support. It seems clear, however, that such elationships play an important role in determining the structure of ne teeth. The evidence that many hypoplastic teeth remain free from aries seems to point clearly to other influences as of more significance.

The Prevention of Tooth Decay.—A fair appraisal of the evidence vailable seems to lead to the conclusion that the view of Bunting nd his associates rests upon stronger evidence than does any other neory. It is their opinion that an acid-forming mouth flora, flourishing n great numbers, is the principal cause of dental caries. According o this view the structure of the teeth is of little importance in elation to caries susceptibility.

There are certain puzzling problems which remain to be solved. One of these is why certain individuals harbor an aciduric flora while thers do not, even when the organisms are persistently implanted in ne mouth. The observations of Bunting's group suggest a possible mmunity to these organisms due to bactericidal or bacteriostatic subances in the saliva or mucus in caries-immune persons which hold nese organisms in abeyance. If such immunity is possible one of the nost pressing research problems relating to public health is the disovery of a vaccine which will immunize people against *L. acidophilus*.

If this theory of the etiology of dental caries is sound one would xpect to find a high incidence of caries in pastoral peoples who ubsist largely upon sour milk. There is an extensive literature suporting the view that sour milk drinking tends to the establishment f an acidophilus flora in the colon, and as pointed out above, Hadley nd associates ('30, l.c.) could find no cultural characteristics which arrant separation of the intestinal and oral acidophilus organisms nto 2 groups. Why do not these milk drinkers develop a profuse ral flora of the strong acid formers? Such evidence as is available ndicates that excessive dependence upon milk as a food does not redispose to excessive caries of the teeth. The excessive milk users at relatively little cereal foods and do not, therefore, take a diet which ould favor the formation of deposits of fermentable material in agnation areas. This is probably of importance.

Orr and Gilks ('31) found dental caries to be less prevalent among ne Masai, who drink milk freely, than among the Kikuyu, who are

largely vegetarian. One is reminded of the finding of Smith, Perso and Harvey ('37) that dogs suffering from multiple deficiencies, h primarily a deficiency of vitamin A, and dogs deficient primarily the blacktongue factor, showed profuse overgrowth of the mouth fusospirochetal organisms, and that recovery from these deficien states was attended by clearing up of mouth conditions (Chap XXI). Presumably this result was determined by humoral facto residing in the mucus, or saliva, or in the superficial epithelial ce of the mouth, which in health are capable of holding in check forn of organisms which may overgrow the debilitated membranes.

Another puzzling problem is why sugar eating is so effective promoting the growth of aciduric organisms in the mouth in co parison with cooked starch eating. Cooked starch is pasty and adhere to the teeth and the diastatic ferments of the saliva would certain form from retained starchy particles in a stagnant area a consta supply of fermentable sugar. One would expect cooked starch eati to be more caries-producing than sugar, since sugar is so soluble th it can hardly be expected to remain long in the mouth. It is of cour easy to visualize how sugar-saturated saliva would remain in pits an fissures, and in stagnant areas, but it should do so to a lesser exte than cooked starch. Yet the present available evidence indicates th sugar is far more effective in inducing caries of the teeth than starc

Of peculiar interest is the effect of a high-fat, low-carbohydra diet, such as is employed in the control of diabetes, in reducin susceptibility to dental caries. When lodged in areas of stagnatio this type of therapeutic diet, while low in carbohydrate, must sti provide an abundance of this substance for acid formation in pit fissures, or incipient cavities formed by acid erosion. We suggest th another role of fat in the diet, viz., that of greasing the tooth surfa and water-proofing it so that acid is prevented from actually comin into contact with enamel, or dentin when the latter is exposed, ma be important in protecting the tooth against even a high concentr tion of acid at a site of decay or potential decay. It is well know to chemists that many substances become coated with oil in preferen to water when both liquids are simultaneously present. One ma visualize the saturation of the debris lining a carious cavity, or a p or fissure, with oil derived from food fat. This would tend to remai indefinitely on the caries-susceptible surface, completely water-proofin it and shielding it from contact with acid arising from fermentatio of food carbohydrate. This view seems not to have been previousl recorded. Investigations should be made to test its validity.

Researches are now needed to test thoroughly the imposing arra of evidence in support of the view that vitamin D (or its equivalent i sunlight) reduces susceptibility to caries of the teeth through in

ving the effectiveness of the body in an immunological sense by itating against the profuse growth of an oral acid-forming and d-tolerating flora. If such an effect could be demonstrated it would far toward reconciling the conflicting data of Bunting and his ociates and those of M. Mellanby, McBeath, and others.

The weakest point in the theory of Bunting lies in the attribution sugar of a greater caries-inducing power than cooked starch, and the assertion that hypoplastic enamel, with its rough surface and s, which from any viewpoint should afford numerous areas of gnation of food residues, is not more likely to favor decay than es normal enamel. The present writers are able to suggest only e possible way in which starch might exert a protective action ainst acid erosion. It is on the basis that starch might be able to sorb considerable amounts of fermentation acid, thus holding it ay from contact with the enamel surface. There is at present no perimental evidence bearing on this point.

It is clear that if we were all to turn to a carnivorous diet, which impracticable or impossible in most countries, tooth decay would appear. It seems that were we to turn to a low-sugar, high-fat type diet, such as is prescribed for diabetic patients, we might expect a mpt and marked reduction in caries-susceptibility. This type of t is practicable in many countries, but fats are in many regions nsiderably more expensive to produce than are starches and sugars. any rate we now know how to produce good teeth as respects ucture and how to preserve them in considerable measure from cay. We may confidently expect that further researches will within ew years see complete unanimity of opinion as to the factors which erate to cause caries-susceptibility. Nutritional research has scored great achievement in the field of dental science.

REFERENCES

NTING (1936). Proc. 9th Internat. Dental Congress, Vienna.

LLER (1889). "Die mikroorganismen der mundhöhle. Die örthlichen und allgemeinen erkrankungen, welche durch dieselben hervorgerufen werden." G. Thieme, Leipzig.

ELLANBY, M. (1929-34). "Diet and the Teeth: An Experimental Study." Medical Research Council, Special Reports Nos. 140, 153, and 191. London.

ATT (1933). "Prophylactic Odontotomy." Macmillan Co., New York.

ANZEN (1930). "Public Health Aspects of Dental Decay in Children." School Health Research Monograph No. 3, Am. Child Health Assoc., New York.

ANKE (1933). "Diet and Dental Health." U. of Chicago Press, Chicago.

SBURY, HASTINGS and MORSE (1931). J. Biol. Chem., 90, 395.

Albright, Aub and Bauer (1934). J. Am. Med. Assoc., *102,* 127
Schour, Tweedy and McJunkin (1934). Am. J. Path., *10,* 321.
Schour (1938). J. Am. Med. Assoc., *110,* 870.
Ruffer (1921). "Studies in the Paleopathology of Egypt." U. of
Press, Chicago.
Weld (1795). "Manners and Customs in Philadelphia."
Catlin (1861). "The Breath of Life." Trübner & Co., London.
Hrdlička (1908). "Physiological and Medical Observations Amo
Indians of the Southwestern United States and Northern Mexic
reau of American Ethnology. Bull. No. 34, Washington, D. C.
Price (1934). Dental Digest, *40,* 52, 81, 130, 211, 240.
Ottofy (1908). Dental Cosmos, *50,* 669.
Marshall (1926). Brit. Med. J., *ii,* 399.
Steggerda and Hill (1935-36). J. Dental Research, *15,* 233.
Brekhus (1931). J. Dental Research, *11,* 487.
Klein, McCollum, Buckley and Howe (1930). J. Am. Dental
17, 782.
Hubbell and Bunting (1932). J. Nutr., *5,* 599.
Hubbell (1933). Am. J. Physiol., *105,* 436.
Koehne, Bunting and Morrell (1934). Am. J. Dis. Child., *48,*
Hubbell and Koehne (1934). Am. J. Dis. Child., *47,* 988.
White and Bunting (1935). J. Am. Dental Assoc., *22,* 468.
Hadley, Bunting and Delves (1930). J. Am. Dental Assoc., *17,* 204
Hadley and Bunting (1932). J. Am. Dental Assoc., *19,* 28.
Jay, Crowley and Bunting (1932). J. Am. Dental Assoc., *19,* 265.
Jay, Crowley, Hadley and Bunting (1933). J. Am. Dental Asso
2130.
Koehne and Bunting (1934). J. Nutr., *7,* 657.
International Institute of Agriculture (1936). League of Nations St
of Food Consumption and Prices, *4,* 26.
Miller and Neuwirth (1935). Dental Cosmos, *77,* 453.
Chase (1926). Anat. Record, *33,* 357.
Bibby and van Huysen (1933). J. Am. Dental Assoc., *20,* 828.
Enright and Friesell (1933). J. Am. Dental Assoc., *20,* 897.
Simonton and Jones (1927). J. Am. Dental Assoc., *14,* 984.
Boyd and Drain (1926). J. Am. Med. Assoc., *87,* 1020; id. (1928
1867.
Boyd, Drain and Nelson (1929). Am. J. Dis. Child., *38,* 721.
Boyd, Zentmire and Drain (1933). J. Dental Research, *13,* 443.
Boyd, Drain and Stearns (1933). J. Biol. Chem., *103,* 327.
McBeath (1932). J. Dental Research, *12,* 723.
Boyd and Nelson (1926). J. Am. Dietet. Assoc., *2,* 45.
McBeath and Zucker (1938). J. Nutr., *15,* 547.
Hanke (1931). J. Nutr., *3,* 433.
Höjer (1924). Acta Paediat., Suppl., *3,* 8.
McCollum, Simmonds, Kinney and Grieves (1922). Bull. Johns H
Hosp., *33,* 202.
Toverud (1936). Suppl. to Den Norske Tandlaege-Forenings Tide

VE (1927). J. Am. Dental Assoc., *14*, 1864.
PERT, WEBBER and CANNIFF (1932). J. Dental Research, *12*, 161.
IN and McCOLLUM (1931). J. Dental Research, *11*, 745.
and GILKS (1931). "The Physique and Health of Two African
Tribes." Med. Research Council, Studies of Nutrition, London.
TH, PERSONS and HARVEY (1937). J. Nutr., *14*, 373.

CHAPTER

XXVIII

Diet in Relation to Healthful Longevity

IN PAST AGES, as well as the present, thoughtful essays h
been written which diligently discussed the problem of prolong
human life (Minot, '08; Metchnikoff, '10; Rolleston, '22; Pearl, '
Robertson, '23; Warthin, '29; Dublin and Lotka, '36). Man's inte
in longevity is indicated also by the constant occurrence of ne
items in the daily newspapers which report panaceas for aging read
furnished to eager news-gatherers by men and women who happ
to reach a ripe old age. Some factors commonly regarded in th
accounts as important in the prolongation of life are diet, alcoho
beverages, tobacco, coffee, work habits, and sleep. But isolated repo
have scarcely any scientific validation. In order to assess the role
any factor in prolonging life it is necessary to make a large numb
of authentic observations in which other factors are statistically co
stant, i. e., have no influence on the variable being measured. Ob
ously, therefore, it is practically impossible to learn the probable eff
of important factors through personal inquiries directed to old perso
Moreover, there are no available records on human beings fro
which statistical analyses can be relied to answer many of the s
nificant dietary questions regarding human longevity. We are ab
from reference to life tables, to predict approximately how ma
persons born in a given year will be alive at some given time in t
future. But we are unable to state definitely, on the basis of any da
what influence several different dietary factors will have on the p
longation of life. For example, we do not know with certain
whether it favors longevity to retard a child's growth through fo
restriction, as suggested by some data to be discussed subsequent
or whether the growth of children should be made to proceed at
rapid rate.

The evidence unequivocally suggests that dietary practices do ence the span of man's healthful existence, but our preoccupation this factor in longevity should not be construed as an indication we regard other factors as without much importance. Space, the general scope of this book, preclude references to other sig- ant factors in determining the length of life. In the discussion to w it shall be our purpose simply to review the most pertinent from animal experimentation which we regard as having suffi- t bearing on human longevity to warrant consideration and to ent our interpretation of the present evidence. Since most of us more interested in healthful longevity than in mere extension of life span through a long period of immaturity and senescence, the hasis shall be on the role of diet in extending the number of s of well-being during maturity.

Prolongation of Life Through Retardation of Growth Rate.— shown by Osborne and Mendel ('15), the capacity of rats to grow ot necessarily lost at the end of the period at which growth ordi- ly ceases. Young rats restricted for months to diets low in certain ntial amino acids grew at a very slow rate, but when the diet made to yield a generous amount of these amino acids rapid wth occurred. Also, resumption of growth was accompanied by capacity to reproduce. At the time of these studies it was believed such retardation of growth occasioned no impairment of the vidual. But Jackson's ('36) investigations indicated that prolonged ting, by means of protein deficiency, causes permanent injury. In ter study Jackson ('37) investigated the effect on growth of rie restriction and noted that refeeding did not cause a full re- ry since there was permanent stunting. Unfortunately it is im- ible to clearly interpret his data owing to the use of a stock diet posed of natural foods for the control animals, while the ex- mental animals were restricted to low-protein or low-calorie diets posed of purified ingredients.

There are, of course, various ways in which growth may be re- ed by dietary means. Although some writers of past generations eved that slow growth promoted longevity only a few attempts e been made to test this hypothesis. The more recent investiga- s made by McCay and associates ('35, '38) require considerable ussion. In studies of the nutritional requirements of brook trout (McCay and Crowell, '34) were led to postulate that "an indi- ual in a species that grows to maturity slowly will have a greater span than one that grows rapidly. . . ." On this basis they ques- ed the wisdom of inducing rapid growth in infants and children. s little wonder, therefore, that their investigations have aroused interest of biochemists and pediatricians.

They have completed two investigations, but at this date (e summer of 1938) only the first one has been published in full this study 106 rats were divided into 3 groups at weaning. basal ration was fed to Group I *ad libitum*. From weaning Grou was allowed to have only enough to permit a gain of approxima 10 gm. per rat at intervals of 2 to 3 months. Group III was gi the basal ration *ad libitum* for 2 weeks after weaning and then stricted in the same manner as Group II. After 766 days on the one-half of the animals in Groups II and III were given the f *ad libitum* and after 911 days the remainder were allowed to all they desired.

In such investigations the composition of the diets obviously very important. Unfortunately it appears that the dietary proced used here was not entirely suitable for the purpose. For instance, ration was entirely devoid of vitamin E and the protein level two times higher than was necessary to promote rapid growth young animals. The composition of the diet was: starch 22, cellu 2, lard 10, sucrose 10, salt mixture 6, dried yeast 5, cod liver oi and unpurified casein 40. Although this same diet was furnished each group of rats, those receiving it in limited amount were gi an additional 3 drops of cod liver oil and 0.5 gm. of yeast per rat d "in order to further compensate for the possible shortage of vitam in the retarded growth groups." Thus complicating factors w introduced which made it difficult to fully interpret the data.

The average life span in days was as follows:

	Group I	Group II	Group III
Males	483	820	894
Females	801	775	826

Twelve animals in the retarded groups were still alive after of those in Group I had died. The greatest life span attained by a animal was 1421 days in Group II. Although the data have not b treated statistically it appears evident that food restriction did p mote longevity.

But when the data are examined it becomes evident that lengthened life span was accompanied by a decided lack of well-bei In fact the restricted animals were practically in a state of chronic validism throughout their unhappy lives. Observations on the sex development, by Asdell and Crowell ('35), showed that in the m severely restricted group the age at first estrus was 357 days, wher in the unrestricted females it was at the 55th day of life. It is diffic

ecide on the basis of the data whether food restriction caused a ngation of the period of regular estrus cycles; but whether it did d not, it is evident that the females allowed to eat *ad libitum* ed a longer period of sexual maturity since they got started 302 ahead of their food-restricted sisters. Moreover, as life waned estricted animals were in an abject state since senility was pro- d. It was estimated that at least one-half of the animals became after they had lived more than 2 years. The bones of the food- cted groups were extremely fragile and "Some crumbled in ourse of dissection."

hus the data indicate that food restriction during the greater of a rat's normal life span promotes longevity, but the existence lorless and in no respect, as yet revealed, does the evidence sug- the desirability of human food restriction, except in ordinary eration, as a means of promoting well-being, and particularly hful longevity.

lealthful Longevity Through Improvement of the Dietary.— ough innumerable observations during the past 30 years of nutri- l investigations have suggested the necessity for well-being of ries abundant in all the various essential nutrients, striking direct nce that healthful longevity is promoted by such regimens has during the past few years from the studies of Sherman and pbell ('24, '28, '30, '35a, '35b, '37). Many generations of rats been raised with success, as judged by moderate standards, in man's laboratory, using a diet of 1 part dried whole milk der, 5 parts of ground whole wheat, and a little sodium chloride. when Sherman and Campbell ('37, l.c.) began to "improve" the by adding calcium carbonate or more milk they obtained "better ts as judged by observations upon growth, adult vitality, and h of life."

he percentage composition of the diets investigated was:

Ingredient	Diet 16	Diet 162	Diet 165	Diet 166	Diet 167
d whole wheat . .	81.97	81.66	77.44	77.14	65.79
milk powder . . .	16.39	16.33	16.41	16.35	16.45
m carbonate . . .	. . .	0.39	. . .	0.38	. . .
r fat	. . .	. . .	4.59	4.58	. . .
milk powder . . .	. . .	. . .	. . .	. . .	16.45
n chloride	1.64	1.63	1.59	1.55	1.31

he following table, constructed from Sherman and Campbell's summarizes most of the significant findings we wish to empha-

Variable Observed		No. 16 No Enrichment	No. 162 Ca as Carbonate	No. 165 Butter Fat	No. 166 Ca and Butter Fat
Gain in gm. during 28th to 56th day of age	male	56.3	71.0	51.0	66.1
	female	47.0	60.2	45.2	57.9
Days age at birth of first young		132	111	134	107
Days duration of capacity to reproduce		213	279	286	241
Young born per female		24.1	32.6	34.2	27.7
Young reared per female		12.7	20.4	19.0	15.3
Average weight of young at 28 days		38.9	42.4	38.9	40.4
Length of life in days	male	658	703	667	689
	female	723	746	818	739

Although we have omitted the statistical analyses of these in the interest of brevity, it is clearly indicated that supplemen of the basal wheat-milk-sodium chloride ration improved the being and longevity of rats restricted to such diets. The basal di apparently deficient in calcium, containing only 0.195 per ce that element. In addition, the content of protein was only 13 cent, and not more than one-third of this was furnished by mi

As stated by Sherman and Campbell ('37, l.c.), "Throughout and early adult life the appearance of animals on diet 167 (ski milk powder replacing part of the wheat in the basal ration) as j by the condition of the coats, and firmness of body was super all others." They noted that in these animals senility began to a at about the same age as in animals receiving the basal ration fied with calcium carbonate alone, butter fat alone, or calciu bonate butter fat. However it progressed more rapidly than i latter groups of animals. Moreover, it was observed that in the groups receiving calcium carbonate and/or butter fat a youthf pearance lasted longer than in the animals receiving the unfo basal ration.

In view of the thesis championed by McCay and associates Cay and Crowell, '34, l.c.), as well as others, that the well-bei individuals is promoted by slow growth during youth, the of Sherman and Campbell offer definite contradiction. Durin 28th to 56th days of life rats receiving the basal ration fortified skim milk powder grew about 40 per cent faster than those rec the unsupplemented diet. The supplemented animals lived l were sexually mature much earlier, reared more young per fe

young weighed more at weaning, and the duration of their capity to reproduce was considerably longer. The findings of Sherman and Campbell confirm the early views of Robertson and Ray) that animals (mice) which grow rapidly live a longer time n slowly growing animals (mice).

The evidence at present suggests indubitably that general well-ng and healthful longevity are promoted, not by food restriction ring youth, but by the generous provision of a well-balanced diet.

Some Problems of Diet in Relation to Healthful Longevity in mans.—It is apparent to the thoughtful person that each experint, in investigations of the nature being discussed, require several rs for completion and is therefore exceedingly expensive. Moreer, the time element renders more likely the introduction of variables ich tend to cause subtle changes in the experimental conditions, s making it difficult to interpret the data. For this reason alone s necessary to be particularly cautious about inferring the desire dietary practices for humans on the basis of observations on mals.

In none of the investigations to date have attempts been made determine the most suitable dietary regimens for different periods life. For instance, it seems rather evident that the amount of tein required during the growth period is different from that ded in adulthood. What are the optimum levels of protein required ing different periods of life? Also, what dietary modifications are eficial in old age to promote clarity of mental processes and edom from crippling physical handicaps? It seems improbable t a diet which is advantageous to a young person is equally suite when he is old. Aged persons, with years of experience and umulated knowledge, personally can enjoy their attainments and form valuable services to society if their regimen of living can be sonably regulated in such manner as to favor full utilization of powers which the inevitableness of senility gradually throttle to nction. Investigations of the future will attempt to determine more cisely the potentialities of improved dietary practices in promoting lthful longevity.

REFERENCES

IOT (1908). "The Problem of Age, Growth, and Death." G. P. Putam's Sons, New York.

TCHNIKOFF (1910). "The Prolongation of Life." G. P. Putnam's Sons, ew York.

LESTON (1922). "Some Medical Aspects of Old Age." Macmillan and o., New York.

RL (1922). "The Biology of Death." J. B. Lippincott Co., Philadelphia.

ROBERTSON (1923). "The Chemical Basis of Growth and Senescence." Lippincott Co., Philadelphia.
WARTHIN (1929). "Old Age the Major Involution." Paul B. Hoeber, New York.
DUBLIN and LOTKA (1936). "Length of Life: A Study of the Life Tab Ronald Press Co., New York.
OSBORNE and MENDEL (1915). J. Biol. Chem., *23,* 439.
JACKSON (1936). Am. J. Anat., *58,* 179.
JACKSON (1937). Anat. Record, *68,* 371.
MCCAY, CROWELL and MAYNARD (1935). J. Nutr., *10,* 63.
MCCAY, MAYNARD and SPERLING (1938). J. Nutr., *15,* Suppl. 12.
MCCAY and CROWELL (1934). Scientific Monthly, *39,* 405.
ASDELL and CROWELL (1935). J. Nutr., *10,* 13.
SHERMAN and CAMPBELL (1924). J. Biol. Chem., *60,* 5.
SHERMAN and CAMPBELL (1928). Proc. Nat'l Acad. Sci., *14,* 852.
SHERMAN and CAMPBELL (1930). J. Nutr., *2,* 415.
SHERMAN and CAMPBELL (1935a). Proc. Nat'l Acad. Sci., *21,* 235.
SHERMAN and CAMPBELL (1935b). J. Nutr., *10,* 363.
SHERMAN and CAMPBELL (1937). J. Nutr., *14,* 609.
ROBERTSON and RAY (1920). J. Biol. Chem., *42,* 71.

APPENDIX

TABLE I

NUTRITIVE VALUE OF PROTEINS OF CEREALS, LEGUMES, AND VEGETABLES FOR GROWTH OF RATS

* Names of investigators are listed on page 660. † Letters in parentheses refer to notes on page 658.

PROTEIN OR PROTEIN-FOOD	METHOD OF PREPARATION	ASSAY METHOD	INVESTIGATOR*	NUTRITIONAL VALUE		
				Level of protein in test diet *Per cent*	Per cent of absorbed N not eliminated in urine	Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Corn	Presumably whole corn. Method of preparation not stated.	Mitchell N bal.	(15)	10	61.3	
	" " "	" " "	(1)	10	59.6	
	Ground South American yellow corn	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	8	67	
	" " "	" " " "	(3)	5	84	
	Presumably whole corn. Method of preparation not stated.	Mitchell N bal.	(1)	5	72	
	Whole yellow corn finely ground	Mitchell paired feeding	(10)	8		1.58 (d)†
	" " "	" " "	(10)	8		1.55 (d)
	" " "	" " "	(10)	8		

	yellow corn					
	" " "	" " "	(16)	9.9		1.38 (s)
	Ground South African white corn	" " "	(16)	9.0		1.30 (s)
Wheat	Whole wheat ground and dried at low temp.	Mitchell N bal.	(9)	8	67 (a)	
	Whole wheat ground	Mitchell paired feeding	(10)	8		1.99 (d)
	" " "	" " "	(10)	8		1.64 (d)
	" " "	" " "	(10)	10		1.70 (d, f)
	" " "	Osborne and Mendel	(11)	10		2.08 (s)
	" " "	" " "	(11)	10		1.58 (s)
	" " "	" " "	(17)	10		1.4 (f, s)
	" " "	" " "	(17)	8		1.2 (s)
	" " "	" " "	(17)	5		0.6 (t)
	Soft English wheat ground	" " "	(16)	9.7		1.36
	Soft English wheat ground and cooked	" " "	(16)	9.7		1.51
Oats	Not stated	Mitchell N bal.	(1)	5	78.6	
	" "	" " "	(1)	10	64.9	
	"Precooked"	" " "	(7)	5	82	

TABLE I—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Oats (*cont'd*)	Standard brand of rolled oats—hulled	Mitchelled paired feeding	(10)	8		2.52 (d)
	Oatmeal	Osborne and Mendel	(11)	10		2.19 (s)
	"	" " "	(11)	10		1.89 (s)
	Oat groats (hulled)	" " "	(17)	10		1.2 (s, t)
	" " "	" " "	(17)	8		1.4 (s, t)
	" " "	" " "	(17)	5		1.8 (s, t)
Barley	Whole grain ground	" " "	(17)	10		1.6 (s, t)
	" " "	" " "	(17)	10		1.2 (s)
	" " "	" " "	(17)	8		1.9 (s, t)
	" " "	" " "	(17)	8		1.6 (s)
	" " "	" " "	(17)	5		1.7 (s, t)
	" " "	" " "				

Rye	Not stated	Mitchell partial fasting	(5)	10		1.0 (s)
	" "	Osborne and Mendel	(13)	10.1		2.51 (s)
	" "	" " "	(13)	10.0		2.05 (s)
	Whole grain finely ground	" " "	(17)	10		1.3 (s)
	" " "	" " "	(17)	8		1.5 (s)
	" " "	" " "	(17)	5		1.4 (s)
	Whole grain finely ground	" " "	(17)	10		1.2 (s)
	" " "	" " "	(17)	8		1.3 (s)
	" " "	" " "	(17)	5		1.3 (s)
Rice	Not stated	Mitchell N bal.	(1)	5	86.1	
Rice bran	Not stated	Osborne and Mendel	(18)	9.02		1.47 (s)
	" " "	Mitchell N bal.	(31)	10	67	
Patent wheat flour	Standard commercial brand	" " "	(8)	8	52	
	Prepared from soft English wheat	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	7	61	
	" " "	" " "	(3)	6	68	
	Source of flour not stated	Mitchell N bal.	(7)	5	72	
	Commercial brand	" " "	(19)	10		0.88 (s)
	" "	" " "	(11)	10		1.27 (s)
	" "	" " "	(11)	10		1.16 (s)

TABLE I—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Nutritional Value: Level of protein in test diet (*Per cent*)	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) (*Grams*)
"Whole wheat" flour	Not stated	" " "	(7)	5	73	
Wheat bran	" " "	Modified Mitchell paired feeding	(20)	6.6		1.05 (d)
	" "	" " "	(20)	5.3		1.23 (d)
	Commercial brand	Osborne and Mendel	(19)	10		1.71 (s)
	" "	" " "	(19)	10		1.96 (s)
Wheat germ	Not stated	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	7	69	
Cornmeal	"Endosperm" prepared from South American yellow corn	" " "	(3)	7	70	
	Not stated	Osborne and Mendel	(18)	9.02		1.18 (s)
Corn gluten	Prepared from South American yellow corn	" " "	(16)	9		0.0± (s)
Edestin	Not stated	" " "	(30)	8		1.49
	" "	" " "	(30)	8		1.11

Peanuts	Roasted					
	"	" " "	(21)	9.2	55.8	
	Fried Chinese peanuts	" " "	(4)	10.4	59	
Peanut oil meal	Not given	Osborne and Mendel	(18)	9.02		1.45 (s)
Coconut meal	Not stated	Mitchell N bal.	(31)	5	77	
	" "	" " "	(31)	10	58	
Soy bean meal	Beans ground, ether extracted and dried	Mitchell paired feeding	(10)	10		0.19 (d)
Soy bean flour	Commercial preparation called "Edelsoja"	Osborne and Mendel	(22)	8.5		1.64 (f)
	" " "	" " "	(22)	10.6		1.57 (f, s)
	" " "	" " "	(22)	15.3		1.58 (f, s)
Linseed meal	Flaxseed ground, dried at low temp. and ether extracted	Mitchell N bal.	(23)	8.7	78 (b)	
	" " "	Mitchell paired feeding	(23)	8.7		1.55 (e)
Cottonseed meal	Cottonseed ground, dried at low temp. and ether extracted	Mitchell N bal.	(23)	8.4	78 (b)	
	" " "	Mitchell paired feeding	(23)	8.4		1.33 (e)
Navy beans	Beans were steam-cooked	Mitchell N bal.	(1)	10	38.4	
	Beans were soaked, boiled until soft, ground and dried at low temp.	Osborne and Mendel	(11)	10		1.48 (s)
	" " "	" " "	(11)	10		1.32 (s)

TABLE I—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Potatoes	Whole potato used. Method of preparation not stated	Mitchell N bal.	(1)	5	68.5	
	" " "	" " "	(1)	10	66.7	
	Potatoes were boiled, peeled, riced, dried at low temp. and ground	Mitchell paired feeding	(24)	7.9		1.34 (d)
	Guberin used. Prepared by heat coagulation of potato juice	Mitchell N bal.	(25)	8.1	71 (t)	
	" " "	Osborne and Mendel	(25)	8.5		2.0 (f, s)
	" " "	" " "	(25)	10.5		1.66 (f, s)
Sweet potatoes (Ipomea batatas)	Protein prepared by heat coagulation of potato juice and drying of purified preparation at low temp.	Mitchell N bal.	(5)	10	93	
	" " "	Osborne and Mendel	(5)	10		1.5 (s)

	" " "	" " "	(20)	11.7		0.69 (d)
Cabbage (Brassica Pekinensis) .	Protein isolated according to method of Kao (26)	Mitchell N bal.	(5)	10	76	
	" " "	Osborne and Mendel	(5)	10		0.9 (s)
Yeast	Whole yeast used	Mitchell N bal.	(1)	5	85.5	
	Leading brand of baker's yeast in Poland	Osborne and Mendel	(22)	8.4		1.48 (f, s)
Coco	Ether-extracted Hershey's coco	Mitchell N bal.	(27)	8	37	

TABLE II

NUTRITIONAL VALUE OF PROTEINS OF MILK, EGGS, AND MEATS FOR GROWTH OF RATS

* Names of investigators are listed on page 660. † Letters in parentheses refer to notes on page 658.

PROTEIN OR PROTEIN-FOOD	METHOD OF PREPARATION	ASSAY METHOD	INVESTIGATOR*	Level of protein in test diet *Per cent*	NUTRITIONAL VALUE	
					Per cent of absorbed N not eliminated in urine	Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Casein	Not stated	Mitchell N bal.	(1)	5	70.8	
	Purified and heated for 3 days	Chick and Roscoe modified Mitchell N bal.	(2)	4.4–15	47	
	Purified without heat	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	6	76	
	Not stated	Osborne and Mendel	(29)	9.3		1.45 (f, s)†
	" "	" " "	(5)	10		2.6 (s)
	" "	" " "	(5)	10		1.9 (s)
	" "	" " "	(6)	10		1.98 (s)
Lactalbumin	" "	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	7	65	
	" "	Osborne and Mendel	(29)	7.9		3.01 (f, s)

	Commercial whole milk powder		(7)	5	89	
	Roller dried winter milk	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	7	86	
	Not stated	Mitchell N bal.	(1)	10	84.7	
Skimmed milk powder	" "	" " "	(8)	8	85	
	Commercial brand containing some fat	Osborne and Mendel	(6)	10		2.89 (s)
Whole egg	Dried	Mitchell N bal.	(9)	8	93	
	"	" " "	(8)	8	94	
Egg albumin	"	" " "	(8)	8.9	83	
	Not stated	" " "	(5)	10	93	
Beef muscle	Beef was ground, dried, reground, ether extracted and sieved	" " "	(8)	9.4	69	
	Trimmed round steak, ground, dried at low temp., ground and ether extracted	Mitchell paired feeding	(10)	8		2.77 (d)
	" " "	" " "	(10)	20		1.64 (d)
	Trimmed round steak, ground and dried at low temp.	Osborne and Mendel	(11)	10		3.15 (s)
	" " "	" " "	(11)	10		2.55 (s)

TABLE II—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Veal muscle	Ground, dried, reground and sieved	Mitchell N bal.	(8)	7.5	62	
	Trimmed, ground and dried at low temp.	Osborne and Mendel	(6)	10		2.88 (s)
Beef liver	Trimmed, free of visible fat and dried at low temp.	Mitchell N bal.	(12)	8	77	
	" " "	" " "	(12)	16	58	
Liver meal	Not stated	Osborne and Mendel	(13)	10.1		1.97 (s)
Beef spleen	Trimmed, ground and dried at low temp.	" " "	(6)	10		2.75 (s)
Beef brain	" " "	" " "	(6)	10		2.97 (s)
Hog brain	" " "	" " "	(6)	10		2.89 (s)
Calf thymus	" " "	" " "	(6)	10		1.81 (s)
Tripe	" " "	" " "	(6)	10		1.73 (s)
Cracklings (pork)	Fat back rendered at 100° C. Cracklings ground	Mitchell N bal.	(14)	8	25	

	neys, and muscle tissue of adults boiled 10 min., minced and dried at low temp.	modified Mitchell N bal.				
Beef lips	Trimmed, ground and dried at low temp.	Osborne and Mendel	(6)	10		2.29 (s)
Beef cheek meat	" " "	" " "	(6)	10		2.36 (s)
Beef tongue	" " "	" " "	(6)	10		2.85 (s)
Hog tongue	" " "	" " "	(6)	10		2.86 (s)
Tankage	Commercial method of prep. not stated	Mitchell N bal.	(1)	10	31.5	
Beef heart	Trimmed, ground and dried at low temp.	" " "	(12)	8	74	
Beef kidney	" " "	" " "	(12)	8	77	
Pork muscle	Dried at low temp. and partially ether extracted	" " "	(9)	8	74	
Pork tenderloin	" " "	" " "	(14)	8	79	
Pork muscle	Not stated	Osborne and Mendel	(11)	10		2.96 (s)
	" "	" " "	(11)	10		2.46 (s)
Lamb muscle	" "	" " "	(11)	10		3.12 (s)
	" "	" " "	(11)	10		2.48 (s)

TABLE III

SUPPLEMENTARY RELATIONSHIPS BETWEEN PROTEINS, AMINO ACIDS, AND PROTEIN-FOODS FOR GROWTH OF RATS

* Names of investigators are listed on page 660. † Letters in parentheses refer to notes on page 658.

PROTEIN OR PROTEIN-FOOD	METHOD OF PREPARATION	ASSAY METHOD	INVESTIGATOR*	Level of protein in test diet *Per cent*	NUTRITIONAL VALUE: Per cent of absorbed N not eliminated in urine	NUTRITIONAL VALUE: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Corn	Presumably ground whole corn	Mitchell N bal.	(15)	10	61.3	
Milk	Presumably dried whole milk	" " "	(15)	10	84.7	
Corn and milk (protein ratio 3 to 1)		" " "	(15)	10	75.7 (g)	
Tankage	Commercial product	" " "	(1)	10	31.5	
Corn and tankage (protein ratio 1 to 1)		" " "	(15)	10	60.0 (h)	
Corn	Whole yellow corn ground	Mitchell paired feeding	(10)	8		1.58 (d)†
	" " "	" " "	(10)	8		1.55 (d)
Corn and lysine (0.25%)	" " "	" " "	(10)	8		1.78 (d)
Corn and lysine (0.15%)	" " "	" " "	(10)	8		1.82 (d)

Corn, lysine (0.15%), and tryptophane (0.13%)	" " "	" " "	(10)	8		2.07 (d)
Cornmeal	Source of cornmeal not stated	Osborne and Mendel	(18)	9.02		1.18 (s)
Peanut oil meal	Source of peanut oil meal not stated	" " "	(18)	9.02		1.45 (s)
Cornmeal and peanut oil meal (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.21 (s)
Cornmeal and peanut oil meal (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.46 (s)
Cornmeal and cottonseed oil meal (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.23 (s)
Cornmeal and soybean oil meal (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.76 (s)
Cornmeal and rice bran (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.63 (s)
Rice bran	" " "	" " "	(18)	9.02		1.47 (s)
Cornmeal and beef muscle (protein ratio 1 to 1)	Apparently commercial cornmeal. Beef was round steak trimmed, ground and dried at low temp.	Osborne and Mendel	(11)	10		3.16 (s)
Beef muscle		" " "	(11)	10		3.15 (s)
Wheat	Hard spring wheat ground	" " "	(11)	10		2.08 (s)

TABLE III—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Wheat and beef muscle (protein ratio 1 to 1)		" " "	(11)	10		3.22 (s)
Wheat and beef muscle (protein ratio 2 to 1)		" " "	(11)	10		2.68 (s)
Wheat	Wheat was ground	Mitchell paired feeding	(10)	8		1.99 (d)
Wheat and cystine (0.25%) . .	" " "	" " "	(10)	8		1.91 (d)
Wheat	" " "	" " "	(10)	8		1.64 (d)
Wheat and lysine (0.25%) . . .	" " "	" " "	(10)	8		2.05 (d)
	" " "	" " "	(10)	10		2.16 (f, d)
Wheat	" " "	" " "	(10)	10		1.70 (f, d)
Patent wheat flour and beef muscle (protein ratio 2 to 1)		Osborne and Mendel	(11)	10		2.67 (s)
Patent wheat flour and beef muscle (protein ratio 1 to 1)		" " "	(11)	10		2.95 (s)
White wheat flour and dried lean beef muscle (protein ratio 2 to 1)		Mitchell N bal.	(8)	8.8	73 (o)	

White wheat flour and dried veal muscle (protein ratio 2 to 1)			(8)	8	52 (l)	
White wheat flour and skim milk powder (protein ratio 2 to 1)		" " "	(8)	8	71 (m)	
White wheat flour and dried egg albumin (protein ratio 2 to 1)		" " "	(8)	9.25	66 (l)	
White wheat flour and whole dried egg (protein ratio 2 to 1)		" " "	(8)	8	75 (j, k)	
Oatmeal	Standard brand of rolled oats	Mitchell paired feeding	(10)	8		2.52 (d)
Oatmeal and lysine (0.25%) . .	" " "	" " "	(10)	8		2.66 (d)
Oatmeal and beef muscle (protein ratio 1 to 1)	Round steak was ground and dried at low temp.	Osborne and Mendel	(11)	10		2.52 (s)
Oatmeal		" " "	(11)	10		1.89 (s)
		" " "	(11)	10		2.19 (s)
Oatmeal and beef muscle (protein ratio 1 to 1)		" " "	(11)	10		3.13 (s)
Oatmeal and beef muscle (protein ratio 2 to 1)		" " "	(11)	10		3.25 (s)
Oats and gelatin (protein ratio 1 to 3)	Method of preparing oats and gelatin not stated	Mitchell N bal.	(15)	10	42.6 (i)	
Rye	Presumably whole ground rye	Osborne and Mendel	(13)	10.1		2.51 (s)

TABLE III—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Liver meal		" " "	(13)	10.1		1.97 (s)
Rye and liver meal (protein ratio 2 to 1)		" " "	(13)	11.4		2.41 (s)
Rice	Not stated	Mitchell N bal.	(1)	5	86.1	
Beef muscle	Round steak trimmed, ground and dried at low temp.	Osborne and Mendel	(11)	10		2.55 (s)
Rice and beef muscle (protein ratio 1 to 1)		" " "	(11)	10		2.50 (u)
Soy bean meal	Beans ground, ether extracted and dried	Mitchell paired feeding	(10)	10		0.19 (d)
Soy bean meal and cystine (0.25%)	" " "	" " "	(10)	10		0.90 (d)
Navy beans	Soaked, boiled, ground and dried at low temp.	Osborne and Mendel	(11)	10		1.48 (s)
Navy beans and beef muscle (protein ratio 1 to 1)	" " "	" " "	(11)	10		2.32 (s)

Garden peas and cystine (0.24%)	" " "	" " "	(24)	8.7		1.62 (d)
Potato (Ohio red)	Boiled, peeled, riced, dried at low temp., and ground	" " "	(24)	7.9		1.34 (d)
Potato and cystine (0.24%) . .	" " "	" " "	(24)	8.1		2.00 (d)
Potato and beef muscle (protein ratio 1 to 1)	Potatoes were peeled, boiled, ground and dried at low temp.	Osborne and Mendel	(11)	10		1.84 (s)
Beef muscle	Round steak trimmed, ground and dried at low temp.	" " "	(11)	10		2.55 (s)
Pork crackling	Fat back rendered at 100° C. Cracklings ground and ether extracted	Mitchell N bal.	(14)	8	25	
Pork tenderloin	Meat was ground, dried at low temp. and ether extracted	" " "	(14)	8	79	
Pork crackling-tenderloin (protein ratio 1 to 3)		" " "	(14)	8.4	72 (p)	

FOOTNOTES TO TABLES ON NUTRITIONAL VALUE OF PROT AND PROTEIN-FOODS FOR GROWTH OF RATS

(a) The practice of discarding unconsumed food residues as was empl here may introduce errors of varying magnitude in the assay of protein of cor food materials such as cereals unless they are very finely ground. The considerable difference in the quality and quantity of protein in various of cereal grains. Hence, when the test animal is allowed to select the pa likes best, variability is introduced which may cause erroneous results. This been critically discussed by Osborne and Mendel (*J. Biol. Chem.*, *34*, 521) Mitchell (*J. Biol. Chem.*, *58*, 873).

(b) In the interpretation of these results it may be of significance that test diet apparently was not sufficiently adequate with respect to the vitami complex for, as the author states "In the next to the last transition period, rat was given daily 0.5 gm. of Northwestern Yeast Company's dried yeast u within one day of the start of the following collection period, in a further atte to stimulate food consumption."

(c) Calculated from data given in paper. It should be noted that th data, secured by a paired feeding procedure, indicate a higher biological va for linseed meal than is given for cottonseed meal. However, data secured the nitrogen balance method (b) suggest no difference between these prote The explanation is that a greater percentage of the linseed meal protein is dig ible. The nitrogen balance method is concerned only with the nutritional va of absorbed nitrogen. Appreciation of this fact is important in evaluating protein feeding value of foods on the basis of values given here.

(d) Calculated from data given in paper.

(f) The significance of these average values is somewhat in doubt owing the lack of adequate numbers of test animals.

(g) According to Mitchell's calculations the biological value of this mixt of protein foods would be 67.2 if there were no supplementary relationshi Since the actual value is 75.7, by determination, it is evident that a supplementa action occurs when corn and milk are fed together.

(h) Since the biological value of a mixture of corn and tankage (1 to 1) scarcely different from that of corn alone (61.3), while that of tankage is or 31.5, a supplementary relationship exists between the proteins of these feeds.

(i) Since gelatin is an incomplete protein it is not possible to decide, on t basis of these and other available data, whether a supplementary relationsh exists.

(j) There is considerable variation between individual values, ranging fr 62 to 92.

(k) It appears that a distinct supplementary relationship exists, since t computed value, if no supplementing effect exists, is 66.

(l) A supplementary action is scarcely indicated, since the computed valu if no supplementary effect exists, is 64.

(m) The value to be expected if no supplementary relation exists is 62, contrast to the actual value of 71.

(n) The value to be expected if no supplementary relation exists is 53, contrast to the actual value of 62.

(o) The value to be expected if no supplementary relation exists is 64, contrast to the actual value of 73.

(p) Since the nutritional value of pork tenderloin is approximately 79 a that of pork cracklings is about 25, it is indicated that a supplementary relatio ship exists.

(q) It may be of considerable significance that corn supplemented with or tryptophane appears to be less capable of promoting growth than unsupp mented corn. Mitchell and Smuts note this apparent growth-depressing effe

yptophane but suggest no explanation of it. Perhaps the growth relation- between corn and tryptophane should be further investigated.

r) Although these data on the supplementary relationships between alfalfa meal and wheat bran appear to indicate a supplementary effect, it should be d that the protein levels of the various diets compared were not comparable. example, in one series wheat bran is fed at a level of 6.6% protein in the diet e the mixture of alfalfa leaf meal and wheat is fed at a level of 8.8% protein that of alfalfa leaf meal alone is 11.0% protein.

s) This value may not represent the actual biological value of the protein, mpared with that of protein in other foods, owing to the lack of comparability od intake. The test animals were allowed to eat the diet *ad libitum*. Since e is bound to be differences in the palatability of rations, it is possible that rences in apparent nutritional value may be attributable, in part, to differ- s in food intake. It would seem that the only way to rule out palatability factor in protein assays is to restrict the food intake of test animals to that imals given a standard protein, perhaps casein or egg protein. That would ssitate also the use of a standard basal diet. In this way the nutritional e of all proteins would be determined with reference to a standard arbitrarily ted protein.

t) The significance of this average value is somewhat in doubt owing to the e differences between individual values.

u) The nutritive value of rice protein has not been determined under com- ble conditions.

References

1. Mitchell, H. H. J. Biol. Chem., 1924, *58*, 905-922.
2. Fixsen, M. A. Biochem. J., 1930, *24*, 1794-1804.
3 Boas Fixsen, M. A., and Jackson, H. M. Biochem. J., 1932, *26*, 1933.
4. Pian, J. H. C. Chinese J. Physiol., 1930, *4*, 431-436.
5. Kao, H., Adolph, W. H., and Lin, H. Chinese J. Physiol., 19 141-148.
6. Hoagland, R., and Snider, G. G. J. Agric. Res., 1926, *32*, 67
7. Mattill, H. A. J. Nutr., 1930, *3*, 17-22.
8. Mitchell, H. H., and Carman, G. C. J. Biol., Chem., 1926, *68*, 215.
9. Mitchell, H. H., and Carman, G. C. J. Biol. Chem., 1924, *60*, 620.
10. Mitchell, H. H., and Smuts, D. B. J. Biol. Chem., 1932, *95*, 263
11. Hoagland, R., and Snider, G. G. J. Agric. Res., 1927, *34*, 297-3
12. Mitchell, H. H., and Beadles, J. R. J. Biol. Chem., 1926, *71*, 429
13. Johnson, D. W., and Palmer, L. S. J. Agr. Res., 1934, *49*, 169-1
14. Mitchell, H. H., Beadles, J. R., and Kruger, J. H. J. Biol. Ch 1927, *73*, 767-774.
15. Mitchell, H. H. J. Biol. Chem., 1924, *58*, 923-929.
16. Boas Fixsen, M. A., Hutchinson, J. C., and Jackson, H. M. Bioch J., 1934, *28*, 592-601.
17. Osborne, T. B., and Mendel, L. B. J. Biol. Chem., 1920, *41*, 275-
18. Maynard, L. A., Fronda, F. M., and Chen, T. C. J. Biol. Chem., 1 *55*, 145-155.
19. Murphy, J. C., and Jones, D. B. J. Biol. Chem., 1926, *69*, 85-99.
20. Hoag, J. R. J. Nutr., 1934, *8*, 235-238.
21. Mitchell, H. H., Burroughs, W., and Beadles, J. R. J. Nutr., 1 *11*, 257-274.
22. Kon, S. K., and Markuze, Z. Biochem. J., 1931, *25*, 1476-1484.
23. Braman, W. W. J. Nutr., 1931, *4*, 249-259.
24. Beadles, J. R., Braman, W. W., and Mitchell, H. H. J. Biol. Ch 1930, *88*, 615-622.
25. Kon, S. K. Biochem. J., 1928, *22*, 261-267.
26. Kao, H. Chinese J. Physiol., 1933, *7*, 379-386.
27. Mitchell, H. H., Beadles, J. R., and Keith, M. H. J. Biol. Ch 1926, *71*, 15-31.
28. Markuze, Z. Biochem. J., 1934, *28*, 463-466.
29. Osborne, T. B., Mendel, L. B., and Ferry, E. L. J. Biol. Chem., 1 *37*, 223-229.
30. Osborne, T. B., and Mendel, L. B. J. Biol. Chem., 1916, *26*, 1-2
31. Mitchell, H. H., and Villegas, V. J. Dairy Sci., 1923, *6*, 222-23

TABLE IV

Distribution of Vitamins A, B_1, and D in Foodstuffs*

	International Units per 100 Grams		
	A	B_1	D
and cereal products			
lfa, leaf meal	16,800–17,000	..	..
ley	0	50–100	..
ad — Graham	0	75	..
hole wheat	0	60	..
hite	0	10	..
ckwheat	..	110	..
rnmeal — white	0	65	..
ellow	350–1,000	65	..
ur — Graham	..	110	..
hite patent	0	4–25	..
hole wheat	0–20	110	..
minv — white	0	70	..
ellow	1,200	70	..
tmeal — whole grain	0–25	325	..
repared breakfast	0	60–140	..
ce — brown	..	50–100	..
polished	0	0	..
polishings	0	300–760	..
e — whole	...	100; 136	..
germ	..	300; 750	..
ybean	200–500	200–400	..
heat — whole	0–20	75–250	..
bran	..	100–500	..
germ	400	400–2,200	..
ts			
ples	100	5–40	..
ricots — fresh	7,500	10–15	..
commercial — dried	9,800	..	..
ocados	150–700	40–140	..
nanas — fresh	385	15–50	..
powder	1,400	50	..
ntaloupe	595	10	..
erries	35–1,150	..	..
anberries	28	..	..
ates, commercial	150–196	10–30	0
gs — fresh	80	18	..
dried	50–100	25–100	..
apes	15–90	10	..
apefruit	0	10	..
ives — canned, green	392	..	..
Manzanillo, ripe	350	2	0
Mission, ripe	140	..	..
anges	300–400	10	..

* Various sources of these data have been used, the compilations of Daniel Munsell (U. S. Dept. Agric., Misc. Pub. No. 275, 1937) and that of Boas-en and Roscoe (Nutr. Abstr. & Rev., 7, 823, 1937–38) being especially helpful.

TABLE IV — *Continued*

	International Units per 100 Gra		
	A	B_1	D
Fruits (cont'd)			
Peaches, fresh, white	7	..	..
fresh, yellow	250–2,800	..	..
dried, yellow	2,000–6,300	10–15	..
Pears	8–15	15–30	..
Pineapple	100–115	25	..
Plums	..	40	..
Prunes — dried	1,000–3,500	90	..
Raisins	95	0–50	..
Raspberries	..	..	..
Strawberries	..	..	..
Tangerines	..	..	..
Watermelon	126	9	..
Juices			
Grape, commercial	0	0	0
Grapefruit	0	10	..
Lemon	0	15	..
Orange	95	30	..
Pineapple	50	..	..
Sauerkraut — commercial	0	..	..
Tomato	825	..	..
Nuts			
Almond	..	40–80	..
Chestnut	..	55–90	..
Filbert	..	220	..
Hazelnut	..	110–220	..
Peanut	..	220–325	..
Pecan	250–500	50	..
Walnut	..	60–150	..
Oils			
Black sea bass liver	52,000,000	..	1,000,0
Cod liver	100,000–250,000	0	8,500–25
Corn — refined	0	0	0
Cottonseed	0	0	0
Halibut liver	1,920,000–36,000,000 (varies greatly)	..	120,00
Herring — entire body	4,860–6,400	..	100,0
Olive — refined	140	..	..
Lamprey — liver — river	..	..	5,3
sea	..	..	4,7
Salmon liver — (Chinock)	2,800,000	..	140,0
Sardine — entire body	24,240–270,000	..	8,0
Swordfish, liver	25,000,000	..	1,100,0
Tuna liver (Bluefin)	512,000–8,400,000	..	4,000,0
(Yellowfin)	4,800,000	..	1,000,0
Dairy products			
Butter	1,000–8,500	..	40–15
Cheese — Cheddar	3,500	0	..
cottage	110	..	..
cream	4,850	..	..
Parmesan	1,750	..	..

TABLE IV — *Continued*

	International Units per 100 Grams		
	A	B_1	D
ducts (cont'd)			
(20%)	1,050	..	..
%)	2,400	..	..
- Hen's whole	1,400–4,500	25–60	15–175
s	1,700–8,800	60–125	45–500
Cow's fresh, whole,			
arket	110–600	10–25	0–10
eurized	252	..	..
d, whole	750–2,800	60–75	0
d, skim	25	75–100	0
porated	775	..	..
lensed	775	..	..
ed	6,750	165–1,010	..
nan, whole	200–500	..	..
s			
oke	392	28	..
agus — green	980	100–135	..
ched	0	40	..
— string, fresh	580–1,400	20	..
, fresh	..	50–75	..
, dried	0	125	..
y, canned	75	40	..
	0	5–10	..
bli — flower	10,000	30	..
	40,000	30	..
	2,000	..	..
n	400	60	..
els sprouts	90	25–35	..
age — green	0	25–60	..
ched	2,800–7,000	10–15	..
Chinese	1,800–4,200	25	..
ts — young	7,700	20–60	..
ure	70	60–110	..
lower	2,100	..	..
— green	20	..	..
ched	34,000	100–200	..
	6,300	30	..
d	..	..	..
— sweet	35	30	..
mber	35,000	..	..
elion greens	70	20	..
ant	28,000	..	..
le	42,000	25	..
	..	15	..
abi	..	125	..
s	6,000	10–20	..
e — green	175	10–20	..
berg	750	..	..
naine	0	50	..
rooms	840	5	..

TABLE IV — *Continued*

	International Units Per 100	
	A	B_1
Vegetables (cont'd)		
Onions	0	10–40
Parsley	105,000	..
Parsnips	..	35
Peas — fresh	500–3,000	25–60
dried	1,750–8,500	70
Peppers — green	1,000–1,300	5–10
red (pimentos)	7,700	5–10
red (chili)	12,500	..
Potato	50	10–40
Pumpkin	..	10
Radish	..	16–60
Spinach	35,000	20–70
Squash — fresh	8,000–40,000	20–50
Hubbard	7,000	..
summer	400–1,400	175
Sweet potato	1,000–7,000	10–35
Tomato — mature, green	1,100	15
ripe, red	1,000–2,100	10–40
Turnips — white	0	15–40
yellow	22	15–40
Turnip greens	8,000	25–30
Watercress	..	30
Meat and meat products		
Bacon	22	90
Beef, lean	20–105	25–100
Chicken — dark meat	..	300
light meat	..	50–150
Ham	..	120–190
Heart — beef	..	75
pork	..	36
mutton	..	40
Kidney — beef	800–900	40
mutton	1,155	40
veal	1,150	40
pig	..	340
sheep	..	190
Liver — beef	10,000–41,000	35–150
calf	52,600–159,800	45
chicken	60,150	35–50
lamb	6,700–113,100	..
pork	11,200–36,700	45
Pork — lean	0	25–185
salt	0	25
Fish		
Clams	28	6
Cod	7	40
Haddock	7	20–45
Herring	50	15–45
Oysters	50–280	75

TABLE IV — *Continued*

	International Units Per 100 Grams		
	A	B_1	D
ntinued)			
. . . . .	4,200	70	..
n — canned	28,800	14–50	200–800
nes — tinned	..	30	..
neous			
, Baker's, dried	0	130–450	0
ewer's dried	..	1,200–6,000	0

TABLE V

Ascorbic Acid Content of Foodstuffs

	Mg. per 100 Grams
Cereals and cereal products	
Oats, sprouted, 1 day	2.5
" 9 days	25.0
dry	0; 11
sprouted, 4 days	20
" 5 days	42
Fruits	
Apple, variety unspecified	0.1 to 20
Apricots	0.8 to 16
Avocado pear	7.0; 13.0
Banana	1.0 to 15
Blueberries	5 to 75
Cherry	3.1 to 17
Currant, black	136 to 220.0
" " , juice	132 to 178 *
" red	50
" " , juice	44 *
Fig	2.0
" white heart	2.4
" inner	4.9
" outer	5.7
Cape gooseberry	26.5; 49
Gooseberry	27.6 to 47.0
" juice	27.3 *
Grape, white and purple	1.0 to 40
" juice	3.0 *
Grapefruit, juice	26 to 65 *
Guava	56 to 299
Huckleberry	44
Lemon	14.0 to 66
" juice	25.8 to 70.9
" whole peel	100.0
Lime, juice	16.8 * to 62.5 *
" " , sweet	31.2 and 58.8 *
" " , unripe	68.1 *
" " , ripe, fresh	39.6 *
" " , " old	23.8 *
Loganberry	20.4 to 48.4
Melon	1.6 to 20
" musk	59
" cantaloupe	15; 53
" water	1.0 to 7.0
Mulberry	6.6 and 21
Nectarine	24
Olive	15
Orange, pulp	16 to 47
" " , valencia	38 to 53
" " , navel	52 to 98.5
" juice	22 to 89 *
" " , Californian	63.2 to 70.6 *
" " , Sunkist, old	10 *
" " , " new	51 *
" " , "	32.5 to 64.7 *

* Per 100 ml.

TABLE V — *Continued*

	Mg. per 100 Grams
(*Cont'd*)	
ach	8.0
" Clingstone	8.2 and 11.0
" Elberta	4.5 and 5.7
" Hardstone	8.5
ar, Bartlett	3.6
rsimmon	6.1 to 20
neapple	10.4 to 62.9
" juice	5.9 to 75.9
um	<0.5 to 4.6
" dried prune	1.0
megranate	15.6
ickly pear, juice	16 and 20 *
uince	10 and 16
aspberry	30.5
" juice	20.8 to 32.6 *
rawberry	46 to 77.5
angerine	10 to 36
" juice	10 to 78 *
mond	0; 19.3
estnut, Spanish	32.3; 50
ocoanut	0.4 to 13.4
azelnut	15
alnut	30
ables	
rtichoke, Jerusalem	5.8
sparagus	12
ean, variety unspecified, dry	1.25
" lima	23 to 61
eetroot, fresh	2.7, 10
roccoli	68
abbage	20 to 124.2
" , early summer	55
" , late autumn	30
" , juice	22 * to 93 *
" , Chinese	40
arrot	1.0 to 31
" juice	4.0 *; 23 *
" tops	95
auliflower	19 to 101
elery, stalks	1.0 to 5.7
ucumber	1.0 to 17.8
andelion, leaves	8 and 42
ndive	19
enugreek	136.7 and 140.7
rass, fresh	68; 75.3
orseradish	52 to 160
is, leaves	110
ale	34
ohlrabi	16 to 100
eek	4 to 33
" tops	50
entil, dry	3.0
" sprouted	15.0

* Per 100 ml.

TABLE V — *Continued*

	Mg. per 100 Grams
Vegetables (Cont'd)	
Lettuce	<0.5 to 22
Lucerne or alfalfa, fresh	73 to 380
" " " young plants	310 to 350
" " " flowering stage	225
" " " dried	5.7 to 160
Mint	63
Mushroom	1.9
Mustard leaves	81
" seeds	44
Onion, white	2.6 to 15
" tops	84
" spring	14
Parsley, green	140 to 280.8
" , root	14.0
Parsnip, greens	210 to 216
" , root	5 to 40
Pea, fresh, green	4.8 to 40
" , chick, sprouted	14.2
" , cow, leaves	70 to 190
" , " , seeds	6.7
Peppers, chillies or pimiento, green, fresh	11.7 to 330
Potato	11 to 36
Pumpkin or squash	1.1 to 22
" " " leaves	10 to 160
Radish	20
" pink	16.9
" white	15.0
" leaves	43 to 113.8
Rape, leaves	33; 48.3
" stem	17.8
Rhubarb, stalks	5.9 to 36.7
" juice	27.8 *
" leaves	30
Soy bean leaves	33.3
Spinach	6 to 124
Sprouts, Brussels	71.8 to 146
Sweet corn, young cobs	3.5 to 9
Sweet potato	16 to 91
Tomato	12.9 to 39
" juice	9.2 to 40
Turnip, root	17 to 43.4
" juice	19.1 *
" greens	39 to 120
Water chestnut	3.2
Watercress	24 to 76
Yam	6.1
Dairy products	
Milk, cow's, raw	<0.3 to 2.89 *
" " " pasture fed	1.64 to 2.18 *
" " " stall fed	1.59 to 2.27 *
" colostrum	1.62 to 3.20 *
" curd from cow's milk	0.7 to 1.0 *
" goat's	0.9; 8.5 *
" human	1.2 to 10.8 *

* Per 100 ml.

TABLE V — *Continued*

	Mg. per 100 Grams
Hen's egg, white	0
" " yolk	0
Duck's egg, white	0.3
" " yolk	1.3
Mollusks and Crustacea	
Clams, small white	17
" yellow	9.3
Fish, air, liver	16.0 to 114.4
" " muscle	6.0 to 27.7
Mussels, fresh-water	0.82 to 1.96
Roe, mackerel, soft	4.0
" perch, hard	10.0
" salmon, hard	14.0
Shrimp	2.5
Meat	
Blood, rabbit	2.3
Brain, ox	11.4 to 26.0
" rabbit	15 to 26
Gizzard, chicken	1.7
Kidney, goat	18
" , pig	14
" , rabbit	3.5 to 10
Liver, calf	33
" , chicken	22
" , duck	13
" , goat	26; 73.4
" , ox	24 to 68
" , pig	12 to 38
" , rabbit	8 to 40
" , sheep	25 to 46
Lung, rabbit	10 to 24
Muscle, goat	7
" , rabbit, red	1.1 to 3.4
" , " , white	0.7 to 1.6
" , " , red and white	0.42 to 1.5
" , ox, stomach	15.8 to 20.1
Heart, goat	8
" ox	4.6
" , rabbit	1.1 to 3.0
Pancreas, ox	12.2
Suprarenal, calf	99
" , ox	76 to 185
" , pig	115
" , rabbit	183 to 216
" , sheep	133
Miscellaneous	
Beer	0.2 to 0.5 *
Sauerkraut, canned	8.2; 21
" , juice	25.*
Tea, fresh leaves, Russian	113 to 187

* Per 100 ml.

TABLE VI

RIBOFLAVIN CONTENT OF FOODSTUFFS

	MG. PER 100 GRAMS
Cereal and cereal products	
Barley, unsprouted	0.01
" , sprouted, 1 to 8 days	0.10 to 0.22
Oats, unsprouted	0.02
" , sprouted 2 to 5 days	0.03 to 0.06
Maize	0.01
Wheat, whole	0.06
" , germ	0.033
Fruits	
Apricot, dried	0.057
Banana	0.0075
Lemon, juice	0.003 *
" , peel and residue	0.006
Orange, juice	0.0069 to 0.0089 *
Vegetables	
Cabbage, white	0.05
Carrot	0.02
Grass, fresh	0.05; 0.142
" , dried	0.6
Lucerne, dried meal	0.7
Pea, unsprouted	0.08
" , sprouted 8 days	0.28
Potato	0.0075; 0.01
Spinach, fresh	0.057
" , dried	0.57
Tomato	0.05; 0.071
Animal products	
Milk, cow's whole	0.1 * to 0.3 *
" , whey	0.045 *
Eggs	
Hen's, yolk	0.5 to 0.6
" , white	0.4 to 0.5
" , " dried	1.41
Fish	
Cod, liver	0.053
Eel, whole	0.18
" , " , young	0.37
" , " , full grown male	0.51
" , blood and muscle	< 0.1
" , liver	0.75 to 1.0
Meat	
Ox, blood	0.0025 *
" , brain	0.1 to 0.5
" , kidney	0.8 to 2.0
" , liver	0.1 to 2.4
" , ovary	0.1 to 0.5
" , spleen	0.05 to 0.1
" , suprarenal	0.5 to 1.0
" , heart	0.553
" , lung	0.89
" , muscle	0
Miscellaneous	
Beer, dark	0.029 *
Honey, pine	0.106
Wine, white	0.0081 to 0.0125 *
Yeast, dried, brewers'	1.8 to 3.0
" , " , bakers'	2.5; 3.6

* Per 100 ml.

Illustrations of the Effects of Specific Deficiencies

Fig. 1. Uterus of guinea pig deficient in vitamin A. The insert shows the columnar ithelium which lines the normal uterus. The contrast is striking, since the normal lumnar epithelium has become transformed into keratinized cells. (Figure kindly rnished by Dr. S. B. Wolbach; insert by Dr. R. H. Follis, Jr.)

FIG. 2. Cross section of the kidney of a rat deficient in vitamin A. Note that the pelvis is filled with keratinized desquamated epithelium. (Courtesy of Dr. S. B. Wolbach.)

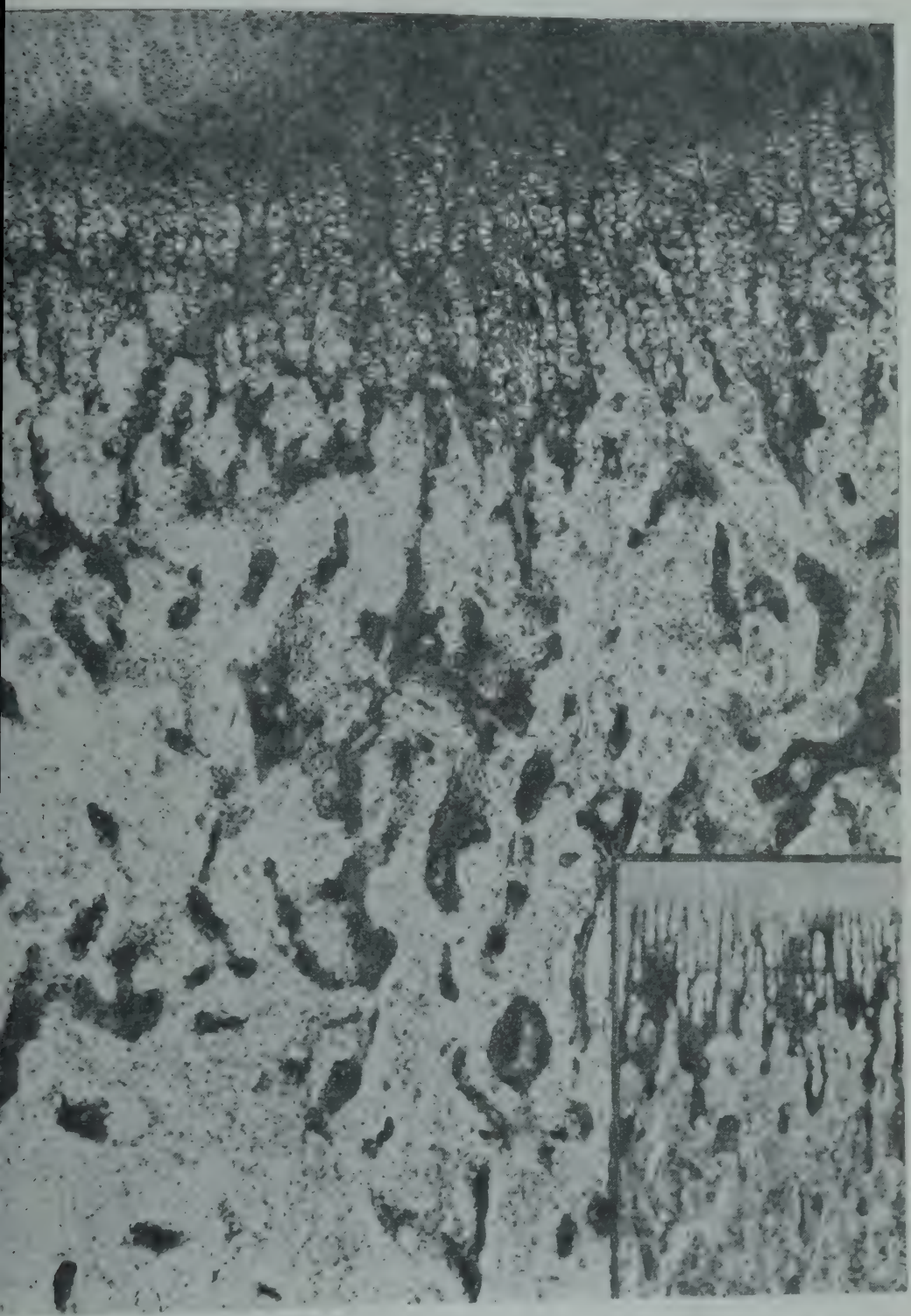


FIG. 3. Rib from an 8 month old child with scurvy. Note the increase of calcified beculae which are easily fractured and consequently do not project vertically as in normal state (compare with insert—lower magnification). There are hemorrhages to the marrow spaces. (Courtesy of Dr. E. A. Park.)

FIG. 4. Transverse section of incisor of guinea pig deprived of ascorbic acid
12 days. Note the marked atrophy of the odontoblastic layer (O) and the retracti
of pulp tissue (P) from the dentin (D). Part of the apparent pulp shrinkage is artefa
(Courtesy of Dr. S. B. Wolbach.)

IG. 5. Transverse section of incisor of scorbutic guinea pig 6 days after restoring rbic acid to the diet. Note that the odontoblastic layer (O) is contiguous with the in (D) and that newly formed dentin is present. The large cavity in the pulp (P) rtefact due to the accumulation of air during the washing process. (Courtesy of S. B. Wolbach.)

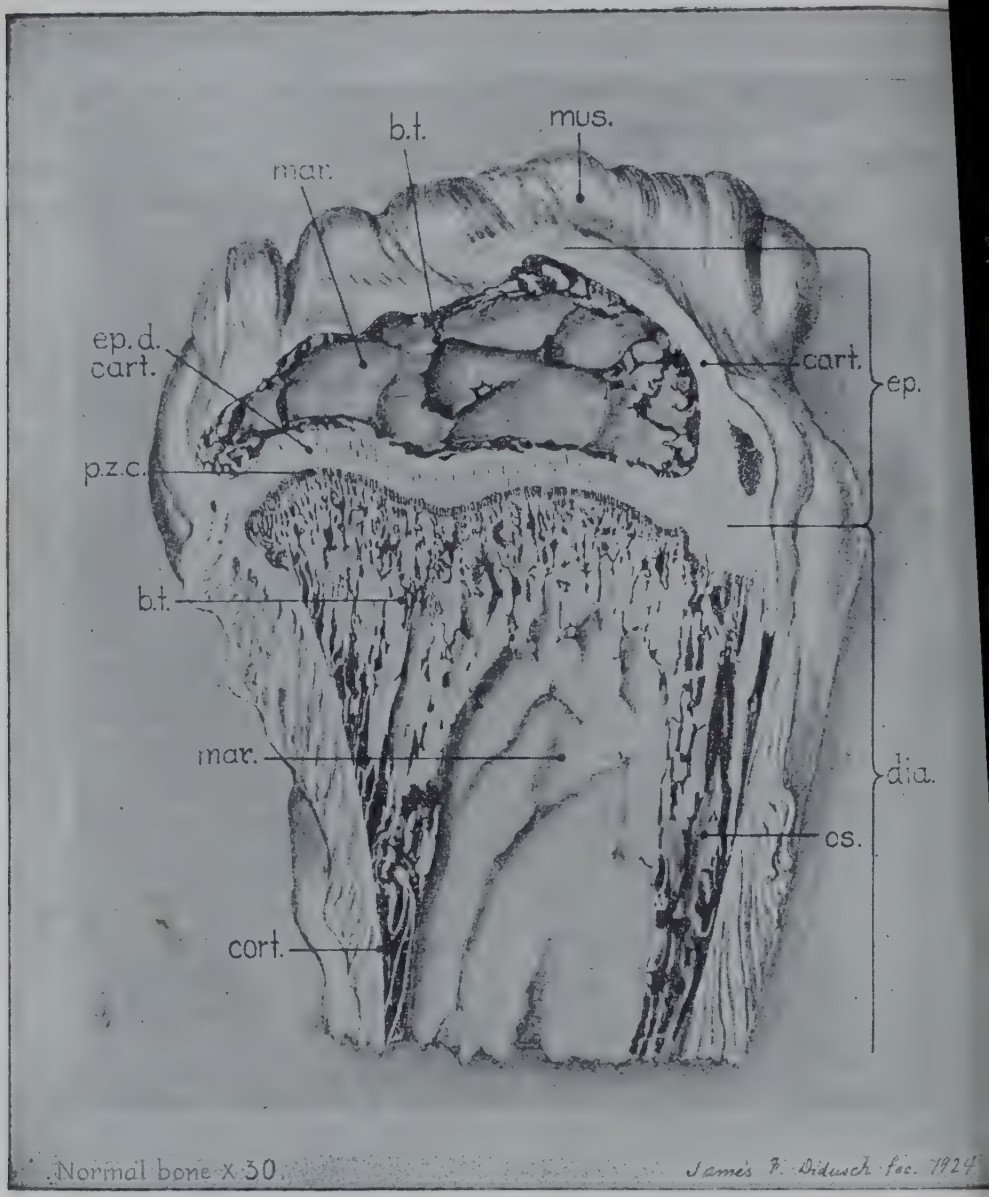
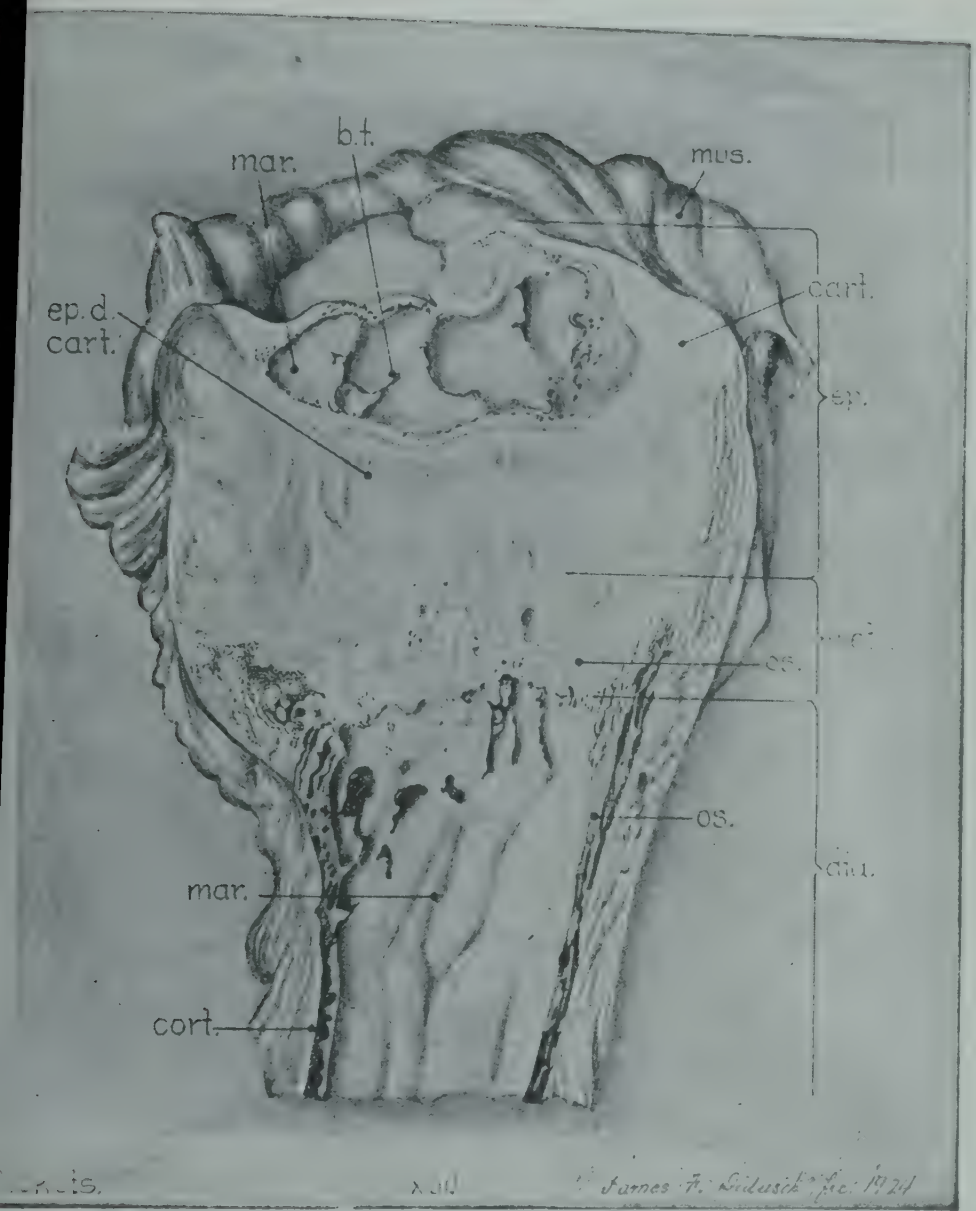


FIG. 6. Normal bone of a rat (proximal end of tibia). This is to be contrasted Fig. 7, which is a ricketic bone, and also with Fig. 8, which shows advanced hea (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of Mead Johnson Biological Laboratory.)

Legend: b.t.—bone trabeculae; cart.—cartilage; cort.—cortex; dia.—diaphysis; e epiphysis; ep.d. cart.—epiphyseal diaphyseal cartilage; mar.—marrow; mus.—mu p.z.c.—proliferative zone of cartilage; os.—osteoid; and met.—metaphysis.



. 7. Bone of a ricketic rat (proximal end of tibia) which shows the florid rickets ed by diet 3143 (high—Ca moderate—P). Note the enormous band of cartilage ne wide metaphysis which is absent from normal bone, and the excessive amount oid tissue. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. n, Jr., of the Mead Johnson Biological Laboratory.)

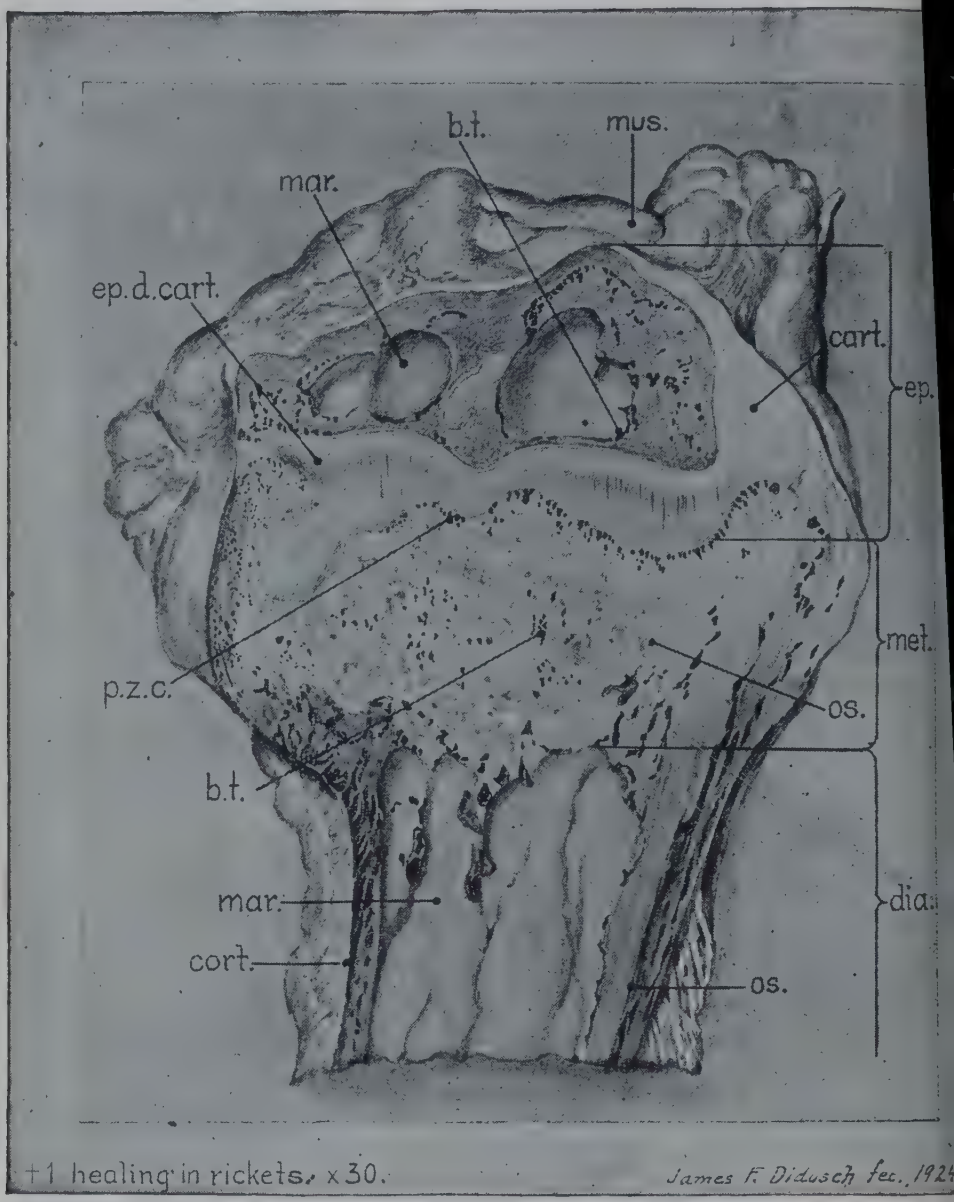
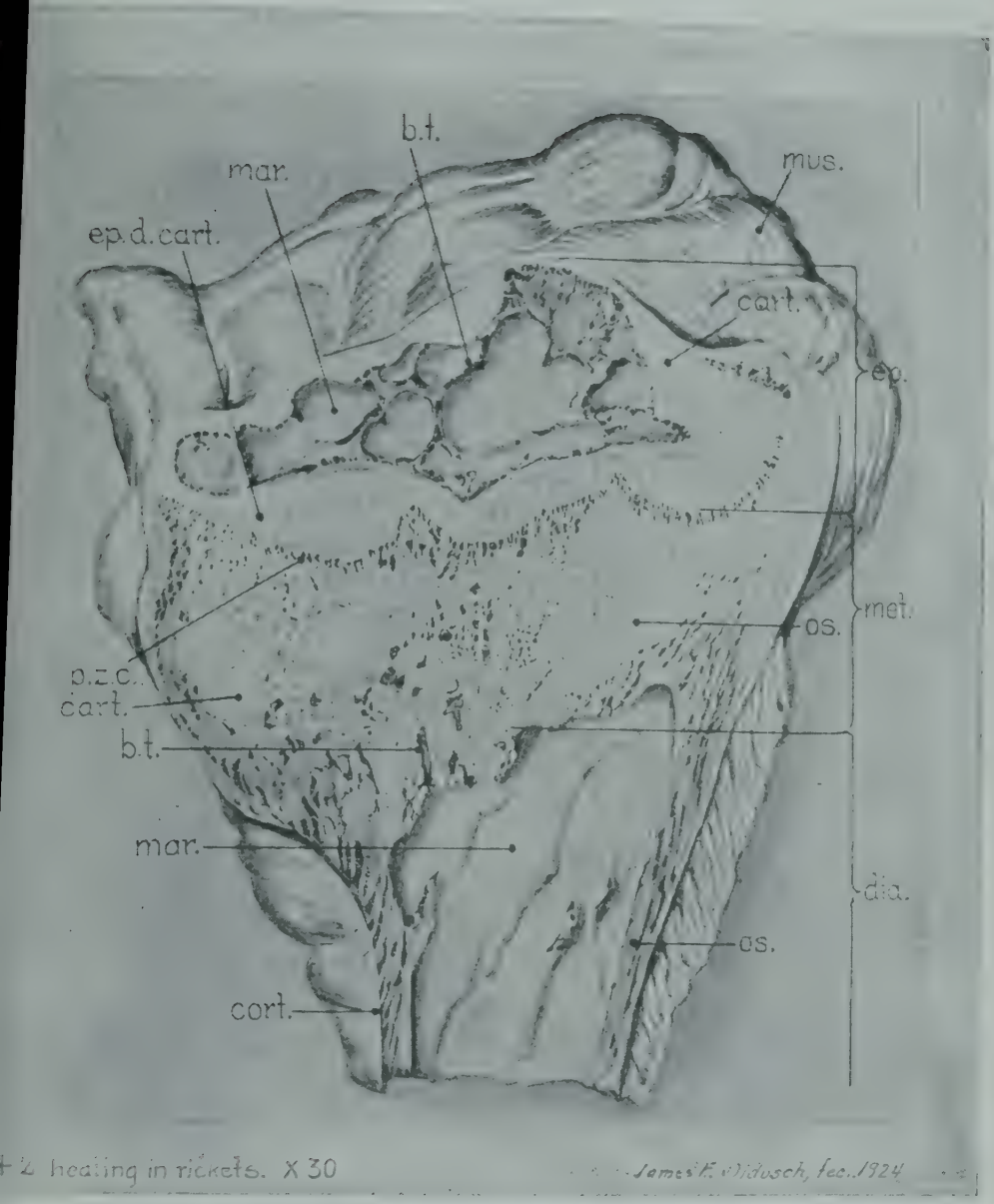


FIG. 8. First stage of healing of a ricketic bone. When vitamin D is admin to a rat whose bones are like that shown in Fig. 7 calcification occurs as sho p.z.c. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnso of the Mead Johnson Biological Laboratory.)



IG. 9. Later stage of healing of a ricketic bone than that shown in Fig. 8. The ication is shown in black at p.z.c. When the administration of vitamin D is con- d the bone returns to the normal structure shown in Fig. 6. If the rickets is e the bone calcifies with deformities. (Drawing by J. F. Didusch, reproduced by esy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

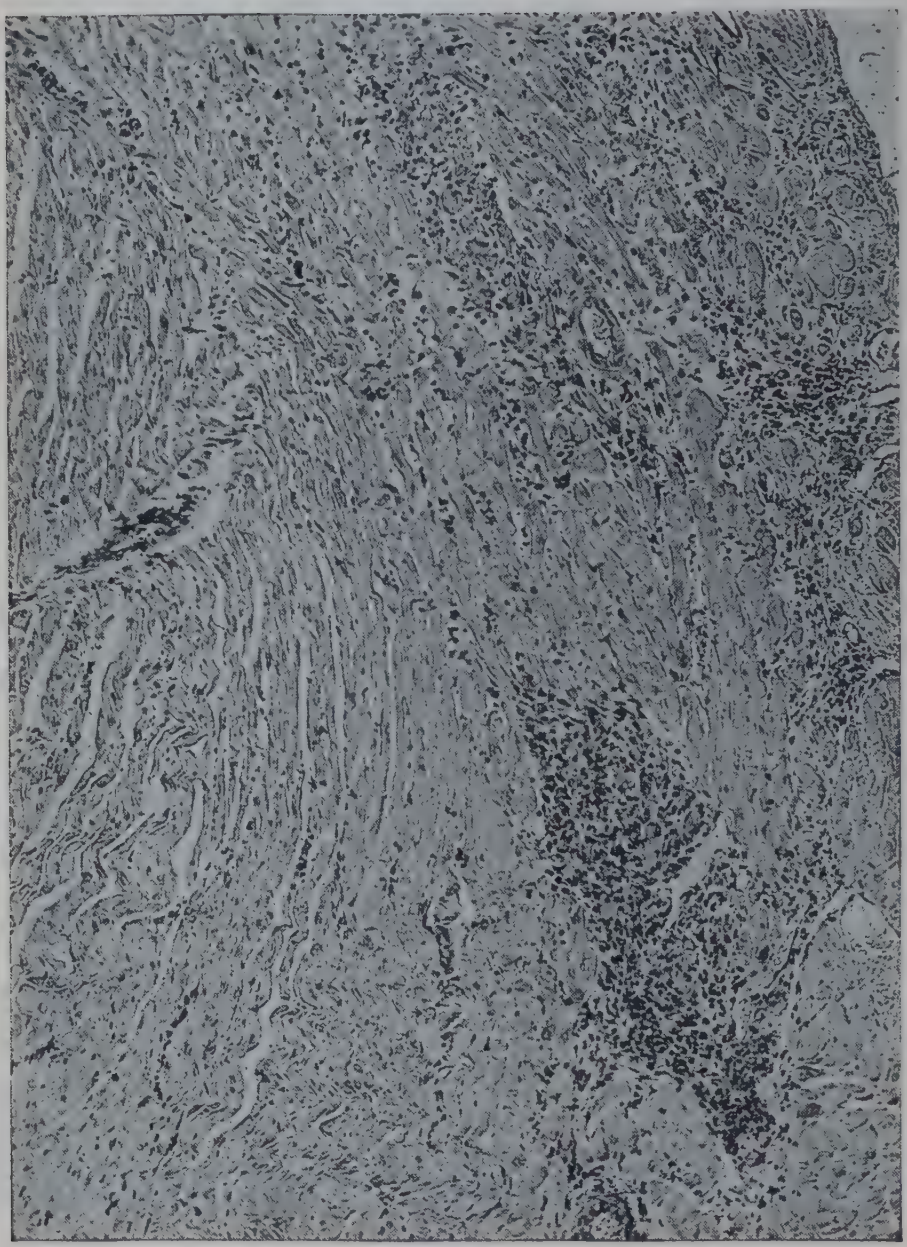


FIG. 10. Heart tissue of rat on a potassium-low diet. Note the foci of leucocytic infiltration where the muscle fibers have become necrotic. Subsequently these areas become replaced by scar tissue. (Orent-Keiles, Follis and McCollum.)

FIG. 11. Typical skin lesions on back of hands and fingers of pellagrin. It appears a deficiency of nicotinic acid is related to the occurrence of pellagra. (Courtesy Dr. MacNeal.)

FIG. 12. Paralysis in hind legs of female rat restricted to a vitamin E-deficient d for approximately two years. (Courtesy of Drs. G. O. Burr, W. R. Brown and R. Moseley.)

FIG. 13. Gross effect of nicotinic acid deficiency in the pig. The basal diet was ground white maize 77.5, peameal 10.5, purified casein 6.5, cod liver oil 3, and salt mixture 2.5. (Biochem. J., *32*, 10, 1938; courtesy of The Biochemical Journal and Dr. H. Chick.)

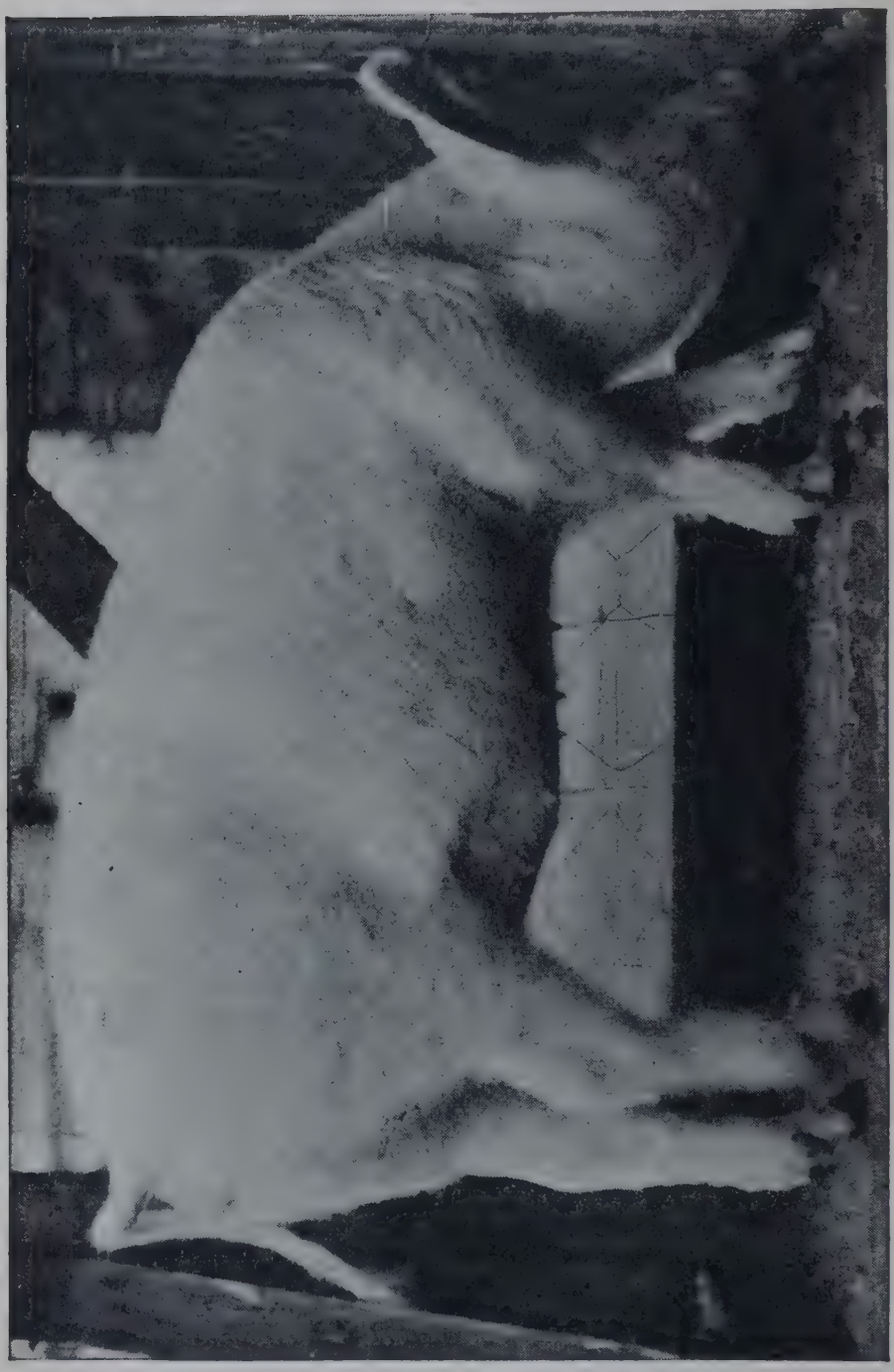


FIG. 14. Pig No. 18 (Fig. 13) three months after receiving 60 mg. of nicotinic acid daily with the basal ration. Note that all symptoms of deficiency have disappeared. (Biochem. J., 32, 10, 1938; courtesy

INDEX

ion, 548, 549
ition, of ascorbic acid, 423
carbohydrates, 32
carotene, 328
fat, 71, 72
vitamin A, 327, 328
vitamin D, 377
of skin in, 375, 376
ctra of carotene, 297
ctra of thiamin, 448
ctra of vitamin A, 298
ctra of vitamin D, 339, 340
nitrile (methyl cyanide), 257
base balance, 169, 196, 424
son's disease, ascorbic acid in, 438
ect of dietary level of sodium and potassium, 205, 206
ine nucleotide, 521
ylic acid, 453
escents, nutritional requirements of, 184, 230, 258, 471
nal glands, content of ascorbic acid in, 422, 424
ation of sodium and potassium to function of, 205, 206
ts, nutritional requirements of, 134, 184-186, 230, 231, 258, 267, 331, 470, 495
ne, 105
pensable in nutrition, 107
mula of, 84
olic polyneuritis, 476-478
fa, 218, 521, 528, 593
inum, absorption of, 276, 277
tribution of, 275, 276
ect of excessive intake of, on phosphorus metabolism, 168, 277
ect of, in perosis, 266
removing fluorine from water, 283
thods of determination of, 276
n-toxicity of, 277, 278
ndin (almond), 89, 105
oblasts, effect of vitamin A deficiency on, 604, 605
e of, in enamel formation, 603
ican Indians, diet of, 588-590
o acid imbalance, effects of, 143, 144
Amino acids, content of, in tissues in relation to composition of dietary protein, 102
dispensable and indispensable in nutrition, 103, 106-111 (*see names of*)
formulae of known, 84-86
glycogen formation from, 39
list of known, 83, 84
methods for isolation of, 87
residues in proteins, 91, 92
Androsterone, relation to vitamin D, 360
Anemia, different kinds of, 211, 213
from deaminized casein, 141, 142, 216
from deficiency of ascorbic acid, 221, 437
from deficiency of B-complex vitamins, 217
from dipyridyl, 219
from exclusive milk diet, 214
from goat's milk, 215
from onions, 216
in domestic animals, 234, 235
microcytic hypochromic, in deficiency of vitamin B_6, 509
physiologic, 233
Aneurin (*see* Thiamin)
Animals, domestic, anemia in, 234, 235, 270, 271
Anorexia, 463, 474-476
Anticalcifying effect of cereals, 168
Antiencephalomalacia factor, 527
Antigizzard erosion factor, 523, 524
Antihemorrhagic factor (*see* Vitamin K)
Antineuritic vitamin (*see* Thiamin)
Antioxidants, 532-534
Antiparalysis factor, for the chick, 519
Antipernicious anemia factor, 237, 238
Antiricketic substances, physical-chemical properties of, 348-351
Antiricketic vitamin (*see* Vitamin D)
Antixerophthalmic vitamin (*see* Vitamin A)
Apatite, 156
Appetite and hunger, distinctions between, 566, 567
Appetite, as guide to selection of food, 569-574

Appetite, effect on mineral consumption, 570, 572
effect on thiamin consumption, 572, 573
for tea, coffee, alcohol, etc., 574, 575
normal and perverted, 566 *et seq.*
Arabinose, 35, 38
Araboflavin, 487, 488
Arachidonic acid, indispensability of, 27, 57, 60
Arginine, 95, 96, 102, 104-106, 141, 144, 238
formula of, 86
relation to histidine, 108
significance of, in nutrition, 108, 109
Arsenic eating, 578
Arthritis, metabolism of sulphydryl compounds in, 115, 116
role of vitamin D in, 389
Ascorbic acid, action of copper on, 226, 405, 407
antigoiterogenic effect of, 258
biological methods of estimating, 409
biosynthesis and origin of, in nature, 412
chemical and physical properties of, 405-407
chemical methods of estimating, 408, 409
compounds physiologically related to, 404
content of, in different tissues, 413, 414
content of, in milk, 425, 426
early views on cause of scurvy, 21, 417
effect in diabetes, 435
effect of cooking and canning on, 407
effects in massive doses, 423
effects of deficiency of, 418-423
enzymatic methods of estimating, 409
enzymes in oxidation-reduction of, 406, 407, 427
estimation of sub-acute degrees of deficiency of, 429, 430
factors affecting retention and excretion of, 423
formula of, 401
functions in body, 426
International Units of, 410
isolation of, 399, 400
limited storage in body, 424, 425
nomenclature of, 398
nutritional significance of, 417 *et seq.*
precursors of, 412
relation to Addison's disease, 437, 438
relation to anemia, 221, 437
relation to cataract, 436
relation to dental caries, 624, 625
relation to gastrointestinal ulcers, 436, 437
relation to infectious diseases, 432-436
Ascorbic acid, relation to vitamin P, 415
requirement of guinea pigs for,
requirement of humans for, 427-4
role of, in immunological reac 430-432
saturation tests for, 429, 430
species-differences in synthesis of, 412
state and function of, in plants, 41
sugars closely related to, 401
synthesis of, 22, 402, 403
Ash, of bone, in rickets, 357, 370
Aspartic acid, 105, 144, 238
dispensable in nutrition, 108
formula of, 84
Athenaeus, 1
Atherosclerosis, experimental, 75
Atwater, 10, 11
Autoclaved yeast, 142, 148

Bacteriophage, protein-nature of, 79
Barley, dietary properties of, 558
vitamin content of (*see Appendix*)
Beans, dietary properties of, 559
vitamin content of (*see Appendix*)
Beaumont, 6
Benzoic acid, 49, 50
Beriberi (*see* Thiamin and Polyneuriti 166, 472, 473
carbohydrate metabolism in, 459, 4 463-465
symptoms of, 459-463
Berries, dietary properties of, 560
Beryllium, 168, 169, 220
Bile, relation to vitamin A absorpti 328
Biliary obstruction and vitamin A ficiency, 328
Biological assay for, ascorbic acid, 409
nutritional value of proteins, 122-1
thiamin, 451, 452
vitamin A, 304-306
vitamin D, 354-357
vitamin E, 546, 547
Bisulfite binding substances (B.B.S.), 4
Blacktongue, and fusospirochetal org isms, 504, 505
and the P-P factor, 505
effect of nicotinic acid on, 512, 513
relation to pellagra, 503, 504
Blacktongue factor (*see* P-P factor), re tion to P-P factor, 512
Blood, composition of, in pernici anemia, 239
in rickets, 356, 369, 370
effect of vitamin D on coagulation 390
hemoglobin level of, 229, 232, 233

d, phosphatase, 370
tamin K in coagulation of, 522
d regeneration, effect of different foods on, 148
d serum, calcium in, 173, 176-180
loride in, 194
rickets, 370, 371
agnesium in, 176-178
osphorus in, 173, 176
dium and potassium in, 194
d vessels, effect on, of deficiency of ascorbic acid, 422
ed flour, 554
c, 168, 173
h, in rickets, 357
lcium, magnesium and phosphorus in, 155-157
ffect of ascorbic acid deficiency on, 421
ffect of calcium deficiency on, 157, 158
ffect of fluorine on, 279-283
ffect of gastrectomy on, 164
ffect of magnesium deficiency on, 162
ffect of mineral deficiencies on, 157-163
ffect of vitamin A deficiency on, 318, 319
ffect of vitamin D deficiency on, 158, 364
nobility of calcium, magnesium and phosphorus in, 180, 181
tructure of, 363, 364
tructure of, in rickets, 364, 365
nemeal, 165
on, 272
n, amino acids in, 106
ietary properties of, 149, 556
itamin content of (*see Appendix*)
ad, dietary properties of, 554, 555
itamin content of (*see Appendix*)
ast-feeding, effect of, in rickets, 385, 386
mine, 273
mobenzene, effect of on utilization of cystine, 113
kwheat, dietary properties of, 558, 559
imia, examples of, 577, 578
ter fat, 73-75, 291
itamin content of (*see Appendix*)

bage, goiterogenic effect of, 256, 257
itamin content of (*see Appendix*)
ciferol (vitamin D_2), 349, 350
cification, pathological, 181, 182
cium, action of vitamin D on utilization of, 170, 171
iological availability, 164-166
ontent in the body, 155, 156, 183
ontent of, in foodstuffs, 187
Calcium, effect of acid-base balance on utilization of, 169
effect of fat on absorption of, 165
effect of, on iodine needs, 251, 256
effect of lactose on absorption of, 43
effects of deficiency of, 157, 158
forms of, in body, 156
human requirement for, 182-184
metabolism in rickets, 370
mobility of, in tissues, 180, 181
relation to milk fever, 158, 159, 390
state of, in foodstuffs, 164
Calcium phosphates, utilization of different forms of, 165, 166
Calcium:phosphorus ratio, 159, 172-174, 185, 219, 220, 371, 372
Calculi, from excess dietary calcium, 182
in vitamin A deficiency, 310, 321-322
Calorimetry, 2, 4, 5, 7, 10
Cancer, effect of amino acid deficiencies on growth of, 115
relation of vitamin E to, 546
Cancerigenic substances, relation of vitamin D to, 358-359
Candy, effect of, on teeth, 615-617, 628
Capillary resistance (fragility) as indicator of nutritional status, 428, 429
Carbohydrate, absorption, 32
cataract-producing action of, 45, 46
conversion into fat, 36
effect on intestinal flora, 42, 43
glycogen formation from, 33-35
in infant feeding, 43, 44
Carbohydrate metabolism, effect of inorganic elements on, 51, 52
effect of thiamin deficiency on, 33, 464, 465, 477
effect of vitamin deficiencies on, 51
nutritional requirement, 52
nutritive value of, 40-45
oxidation of, 33-35
relation to refection, 46, 47
requirement for fat oxidation, 35
role of adrenal cortex in, 38, 39
role of hypophysis in, 37, 38
role of pancreas, 40
selective action on, 32
sex variation in, 39, 40
sodium and potassium in, 204, 205
Cardiovascular dysfunctions, 461, 477
Caries (*see* Dental caries)
Carotenase, 296
Carotene, 291, 293-298
absorption of, 328
absorption spectra of, 297
as source of vitamin A, 292
chemical behavior of, 296, 297
content of various substances, 298

Carotene, conversion into vitamin A, 293, 296, 297
forms of (isomers of), 293
properties of, 297, 298
provitamin A, 292
structure, 293, 294
synthesis, 296
Carotenoid pigments, 295, 303
Casein, amino acids in, 105, 106
deaminized, 141, 142, 216
effect of heat on nutritional value of, 137-141
nutritional value of, 130, 138-141
significance of phosphorus in, 167
Cataract, relation to riboflavin deficiency, 491
role of ascorbic acid in, 436
role of galactose in, 46
role of lactose in, 45
Catatorulin effect, 453, 464, 465
Celiac disease, role of vitamin D in, 388
Cereal grains, anticalcifying action of, 167, 168
general nutritive properties of, 132, 552, 553
nutritive value of proteins of, 131-133, (*see Appendix*)
vitamin content of (*see Appendix*)
Cereal proteins, nutritive value of, 131-133
relative deficiency of, in indispensable amino acids, 104, 105
Cevitamic acid (*see* Ascorbic acid)
Chick dermatitis factor (*see* Filtrate factor)
Chickens, 411, 460, 492, 493, 510, 522-524, 527
anemia in, 235
antiparalysis factor, 519
arginine requirement of, 108
ascorbic acid dispensability in nutrition of, 411
factor U in nutrition of, 521
manganese deficiency in, 265-267
vices in (toe picking, etc.) 576, 577
vitamin B_4 in nutrition of, 519
vitamin K in nutrition of, 523
Children, nutritional requirements of, 134, 183-186, 229, 230, 258, 267, 331, 470, 474, 475, 495
Chinese, diet of, 133, 592, 593
Chittenden, studies on protein requirements, 10-12
Chlorine, effects of deficiency in diet, 203, 204
Chlorophyll, magnesium in, 164
relation to ascorbic acid, 410
Chlorosis, 578
Cholesterilene, 347, 351
Cholesterol, 297, 339, 350
absorption spectra of, 339, 340
antiricketic value of, 340
effect of feeding, 75
effect of irradiation on, 338
in animal tissues, 66
Cholesterol esters, role of choline in utilization of, 63
Cholic acid, effect on sulphydryl compounds, 113
relation to gizzard erosion, 524
Choline, in tissues, 64-66
lipotrophic action of, 62-65, 76
Chondrodystrophy, 102
Chondroitin, 524
Chroman ring, relation to vitamin E, 536-538
Citric acid, 47, 48
Clay eating, 575
Climate, and dietary practices of man, 58
Coagulation, of blood, role of vitamin K in, 522
Cobalt, deficiency of, in domestic animals, 270, 271
distribution of, 269
effect of manganese on toxicity of, 270
polycythemia from excess of, 215, 269, 270, 426
Cocarboxylase, 166, 449, 464-466
Cod liver oil, history of use of, 336, 337
toxicity of, 68-70
vitamin A in, 23, 24, 291
vitamin content of (*see Appendix*)
vitamin D in, 23, 24, 291
Coenzyme, cocarboxylase, 166, 449, 464-466
yellow, 489
Colostrum, 268
Complement, relation to ascorbic acid, 431
Concept of modern nutrition, 28, 29
Convulsions, 157, 161
Copper, availability of, 227
content in foodstuffs, 228
controversies regarding hematopoietic significance of, 214
discovery of its indispensability in hematopoiesis, 28, 213, 214
effect of deficiency on hair pigmentation, 226
effect on ascorbic acid, 405, 407
human requirements for, 228-231
metabolism of, 224, 225
relation between age and content, in tissues, 224, 225
role in hematopoiesis, 225, 226
Corn (*see* Maize)
Coumaran ring, relation to vitamin E, 536-538
Cowgill's formula, 470

nase, and nicotinic acid amide, 514
nism, early views on cause of, 244, 245
ure of, 248
oxanthin, 296, 553
l theory of protein structure, 89, 90
mine, 112, 113
ine, 112, 114
ne, 95, 96, 102, 104-106, 129, 132, 139, 141, 143, 144, 150
mically related compounds, 112
pensability in nutrition, 21, 112, 113
ct on hair and wool growth, 114
tors affecting utilization of, 113
mula of, 85, 112
hrome, 226

adaptation, technic for determination of, 312, 313
inized protein, 141, 142, 216
iency disease, development of concept of, 15-19
ology of, 19
droascorbic acid, formula of, 401
ation to l-ascorbic acid, 400, 405, 406
hydrocholesterol, 344, 347, 359
drocholic acid, 524
hydrositosterol, 346, 351
hydrostigmasterol, 347
al caries, arrest of, by diabetic diets, 619
corbic acid and, 418-421, 624, 625
mposition of saliva and, 612, 613
perimental production of, by diet, 625-627
om destructive deposits, 618
npactation of coarse particles and, 626
cidence of, 608-610
ture of, 600-602
evention of, 627
eories of etiology of, 600-602
tamin A and, 604, 605
tamin D and, 391-393, 622-624
tin, formation of, 606, 607
matitis, in the chick, 510
the rat, 506
lation of factor "Y" to, 519
lation of filtrate factor to, 510
lation of vitamin B_6 to, 506-509
oxication, effect of amino acids on, 115, 116
, in relation to longevity, 632 *et seq.*
relation to the teeth, 600 *et seq.*
nosphorus deficient, 159
roblems of, in relation to longevity, 637
ckets-producing, 354
orbutic, 420
nerman, wheat and milk, 635

Diet, vitamin E deficient, 547
Dietary essentials, biological methods of determining, 21
modern concept of, 15 *et seq.*
summary of present concept of, 29
Dietary habits, effect of modern transportation on, 5, 98
extremes in, 567, 568
in cold regions, 581, 582
in warm regions, 583, 584
of American Indians of the Plains, 588, 589
of Cassava eaters, 584
of Chinese, 133, 592, 593
of desert borderlands, 586, 587
of early Egyptians, 581
of East Congo pigmies, 586
of hunting peoples, 587, 588
of Italians, 595-597
of Japanese, 592
of Javanese, 584, 585
of Labrador, 594
of man in different parts of the world, 580 *et seq.*
of Newfoundland, 595
of primitive forest people, 585
of primitive Pacific Islanders, 590, 591
of reservation Indians, 589, 590
of Slavic peoples, 597, 598
problem in nutrition of young children of Orient, 594
Dietary properties of foodstuffs, 552 *et seq.* (*See names of individual foodstuffs*)
Dieting, 2
22-dihydrocalciferol, 345, 347, 350
Dihydrocholesterol, 340, 344, 350
22-Dihydroergosterol, 345
Diiodotyrosine, 249
Diodorus, 2
Diphtheria, role of ascorbic acid in, 434-436
Dipyridyl, 218, 219
Dog, blacktongue in, 503-505, 512
dispensability of dietary ascorbic acid in, 411
riboflavin deficiency in, 491, 492
Durohydroquinone, 536

Eczema, in infants, 60
Edema, in relation to dietary protein deficiency, 144-147
in relation to thiamin-deficiency, 461
Edestin, amino acids in, 105
"complete" for growth, 105
in phosphorus-deficient diet, 159
Egg albumin, amino acids in, 106
amino acid residues in, 91, 105, 130
molecular weight of, 89
toxicity of, 150, 151

Eggs, content of iron and copper in, 223
dietary properties of, 130-132, 141, 221, 223, 564, 565
effect of diet on composition of, 102
effect of diet on hatchability of, 102, 266, 267
vitamin content of (*see Appendix*)
Egg white, toxicity of, 150, 151, 506
Eijkman, 17, 18
Elaidin, in studies of indispensable fatty acids, 58
Electrocardiograph, 203, 453, 461
Embryo, role of vitamin D in development of, 382
Enamel, destructive deposits on, 618
effect of acid on, 608
formation of, 603-606
protective coating of, 617
Encephalomalacia, in the chick, 527
Endogenous protein metabolism, 82
Energy, dispensability of protein as source of, 79
metabolism, 197
Enzymes, organizers in protein synthesis, 100
protein nature of, 79
Warburg-Christian, 514
Epidermal tissues, growth of, in relation to sulphydryl amino acids, 113-115
Epinephrin, 121
Epithelium, changes in vitamin A deficiency, 313-315
seminiferous, injury to, 323, 324
Erasistratus, 2
Ergosterol, 339, 349, 359
absorption spectra of, 339, 340
antiricketic value of, 340
effect of irradiation on, 340
products of irradiation of, 341-347
Erythrocyte sign, 539
Eskimos, diet of, 582
Estrone, relation to vitamin D, 359
Estrus cycle, 56, 322, 323, 538-540
Endogenous protein metabolism, 82
Extrinsic factor in pernicious anemia, 237, 240
Eyes, effects of sodium deficiency on, 198
effects of vitamin D deficiency on, 389, 390
relation of vitamin A to, 308-313

Factors, filtrate, 510, 511
for cure or prevention of muscular degeneration, 528, 529
for nutrition of lower forms of life, 529
L_1 and L_2, 526
U, 521, 522
Factors, W, 520, 521
Y, 519
Fads, dietary, 12, 563, 564, 568, 56
Fasting, 33, 100, 195
Fat, absorption, 71, 72
dietary, relation to body fat, 71
effect on calcium and phosphorus sorption, 165
effect on carbohydrate utilization,
effects of deficiency on experim animals, 55, 56, 59
effects of deficiency on humans, 60
formation from carbohydrate, 36
in animal tissues, 66, 67
in infant nutrition, 70
in milk, composition of, 72-74
effect of dietary fat on, 74, 75
source of, 72-74
indispensability of, in nutrition, 27, 58
phospholipids, 62, 63
fatty acids in animal tissues, 66, 6
function of, 67
relation of antioxidants to, 533
role in refection, 61, 62
role of thiamin in synthesis of, 46
sparing action on thiamin, 468, 469
Fatty acids, in animal tissues, 66, 67
indispensable, 27, 56, 58
Feathers, vitamin D activity of, 374,
Fetus, calcium and phosphorus in, 156
effect of manganese deficiency on,
effect of vitamin D deficiency on,
effect of vitamin E deficiency on, 5 540, 548, 549
Fibrin, blood, amino acid residues in,
Fiji Islands, diet of inhabitants of, 5 591
Filtrate factor, deficiency of, 510
diets for producing deficiency of, 51
effect on blacktongue, 511
occurrence, 510, 511
properties of, 510, 511
relation to P-P factor, 511
role of, in chick pellagra, 510
Fish oils, vitamin A in, 298, 299 (*Appendix*)
vitamin D in, 352, 353 (*see Append*
Flavin (*see* Riboflavin)
Fletcherism, 12, 568, 569
Flour, amino acids in, 106
wheat, dietary properties of, 131-1 138, 554, 555
Fluorapatite, 156
Fluorine, content in certain rock ph phates, 280
content in water supplies, 281
dispensability in diet, 283
distribution of, 279

e, effects of excesses on teeth, 279
led enamel from ingestion of, 280,
1
ental transference of, 280, 283
val from water, 283
toms of toxicity from, 281, 282
theory of protein metabolism, 81,
nalysis, biological method of, 21
procedures, 8
uffs (*see individual names of food-uff*)
ent of copper in, 225, 227, 228
ent of iron in, 222, 223
t on hemoglobin regeneration, 216
res, healing effect of vitamin D on,
91
lite, 156
noice, in food selection, 569-574
se, 32, 35, 36, 42
general nutritive properties of, 560
min content of (*see Appendix*)
s earth, 443, 450
pirochetes in pellagra, 504, 505

ose, 32-36, 43-45
ct of fat on utilization of, 61
in cataract formation, 45, 46
2
c juice, 237, 238
intestinal disorders, 461, 462, 478,
79
intestinal ulcers, role of ascorbic
cid in, 436, 437
n, 89, 121, 130, 141
no acids in, 105
inal epithelium, effect of vitamin A
leficiency on, 323
ic acid, 297
tmark, 421
rd erosion, 523, 524
in, 105, 141
se, 33, 41
tabolism of, 33-36, 42
e of absorption, 32
tion to ascorbic acid, 401, 403
mic acid, 103, 105, 106
pensable in nutrition, 108
mula of, 84
mine, 108
thione, 144, 406
nposition, 116
tritive significance of, 113, 114
nin, 105
rophosphates, nutritional role of, 168
ne, 50, 102, 105, 238
pensable in nutrition, 107
mula of, 84

Glycogen, 33-35, 37-40, 49, 59
formation from amino acids, 39
formation from carbohydrate, 33-35
storage, 34
Goat's milk anemia, 215
Goiter, antigoiterogenic effect of ascorbic acid, 258
colloid, 248
early views on cause of, 244, 245
effect of acetonitrile on production of, 257
effect of allyl isothiocyanate on production of, 257
effect of cabbage in production of, 256, 257
effect of fat in production of, 256, 257
endemic, 248
exophthalmic, 248
goiterogenic effect of excess calcium, 256
use of iodine in prevention and treatment of, 251-254
Gorgonia flabellum, 95
"Grass Juice" factor, effect on rat growth, 525, 529
occurrence of, 525
relation to reproduction in the guinea pig and rabbit, 525, 526
Grijns, 18
Growth, effect of diet improvement on, 635-637
different levels of protein on, 136
mineral deficiencies on, 157, 159, 161, 201, 203, 262, 268
prolonged protein restriction on, 633
vitamin E deficiency on, 542
relation of vitamin A to, 308, 318
relation of vitamin D to, 382, 383
Guaiacum reaction, 399
Guinea pig, ascorbic acid needs of, 429
goiter in, 258
lack of stored iron at birth of, 213
l-gulose, 401
Gum tissue, effects of magnesium deficiency on, 161
effects of vitamin B-complex deficiency on, 217

Haemocuprein, 227
Hair, effect of copper deficiency on, 226, 227
growth in relation to sulphydryl amino acids, 114, 115
relation to vitamin D, 374, 375
Hay fever, role of vitamin D in, 389
Heart, effect of K deficiency on, 203
effect of thiamin deficiency on, 453, 461

Heat, effect on nutritional value of proteins, 137-141
Hematopoiesis, effect of liver on, 236
effect of vitamin A on, 319, 320
influence of various foods on, 216
Hemeralopia, relation to vitamin A, 309
Hemocyanin, content of copper in, 211
in lower organisms, 28, 211
molecular weight of, 89
Hemoglobin, amino acid composition of, 96, 105
amino acid residues in, 91
effect of pregnancy and lactation on level of, 232-234
Hemophilia, relation of vitamin K to, 523
Hexose phosphates, nutritional role of, 168
Hexuronic acid (*see* Ascorbic acid)
Hippocrates, 2, 6
Histidine, 95, 96, 102, 104-106, 139, 141, 144
formula of, 86
indispensable in nutrition, 109
relation to arginine, 109
replaceability of, by related compounds, 109
Holst and Fröhlich, studies on scurvy, 417
Homocysteine, 112, 113
Homocystine, 112, 113
Homomethionine, 112
Hopkins, 16, 17
Hunger and appetite, distinctions between, 566, 567
Hydrogen sulfide, 227
Hydroxyglutamic acid, 105, 238
dispensable in nutrition, 108
formula of, 85
Hydroxyproline, 105, 238
dispensability in nutrition, 111
formula of, 86
Hyperparathyroidism, effect on bones and teeth, 171, 172, 607
Hypoproteinemia, effects of, 144-147

Immunological mechanisms, relation of ascorbic acid to, 431
relation of vitamin A to, 326
Infantile paralysis, role of vitamin D in, 390, 391
Infantophagia, 576
Infants, fat in diet of, 70, 71
lactose in diet of, 43, 44
nutritional requirements of, 183, 185, 186, 229, 230, 331, 426, 470, 474, 475, 495
Infection, artificially induced, relation of vitamin A to, 325, 326
relation of ascorbic acid to, 432-436
relation of vitamin A to, 324, 325
Inhibitols, 532-534

Inorganic elements in nutrition, sur
of, 27, 28
Inositol, 164, 456
Insulin, molecular structure of, 90
nickel in, 271
zinc in, 268
International adsorbate, 450
International unit, of ascorbic acid,
of thiamin, 450
of vitamin A, 304
of vitamin D, 357
Intestinal flora, role of carbohydrat
42, 43
Intracellular proteinases, 100, 101
Intrinsic factor in pernicious anemia,
Iodine, early experiences with, 27,
246
compounds in body, 249
content in normal and goiterous
roids, 248
content in sea water, 250
effect of calcium on utilization, 25
effect of glaciation on soil content
251
effects of deficiency of, 247
effects of large amounts of, 254, 25
factors in soil that cause depletion
251
geographic area of deficiency,
251
in relation to thyroid function, 244
seq.
requirement of humans for, 258, 25
therapeutic and prophylactic use
251-253
utilization of organic and inorg
forms of, 259
Iodized salt, 253
Iodoacetic acid, effect on sulphydryl c
pounds, 113
Irish moss, dietary properties of, 562
Iron, absorption of, 217, 218
availability of, 218, 219
content in fetus, 234
content in hemoglobin of adult
211
content of available iron in foodst
222-224
early views of role in hematopoi
212, 213
effect of menstruation on requirem
for, 231, 232
effect of pregnancy and lactation
requirement for, 232-234
estimation of ionized form with dip
dyl, 218, 219
human requirement for, 228-234
metabolism of, 221, 222
parenchymal, 222

lization of, effect of the Ca:P ratio, 168, 219, 220
tion of, effect of vitamin A, D, ascorbic acid, 220, 221
per, and nutritional anemias, 211
eq.
on of foods, 353, 354
, 105
la of, 84
nsable in nutrition, 107
diet of, 595-597

, diet of, 592
, diet of, 584, 585

ietary properties of, 562
zation of epithelium, relation to amin A, 309, 310, 313-316
, 95 105
alacia, in vitamin A deficiency, , 310
carbohydrates in production of, 35
effect of, magnesium deficiency on, 163
ess calcium on, 182
h protein diets on, 142, 143
assium deficiency on, 203
amin A deficiency on, 182, 321, 322

umin, amino acids in, 105
t of heat on, 138
tive value of, 130, 138, 142
on, effect of manganese deficiency , 262-265
r L_1 and L_2, 526
tive requirements for, 184-186, 5, 428, 429
acid, 35, 39, 41, 49
cillus acidophilus, 601, 612-615, 7, 618, 621, 625-627
vin (*see* Riboflavin)
, cataract, 45, 46
of fat on utilization of, 61
of, on absorption of calcium, 42,

fant feeding, 42-44
olism of, 33-36, 41-44
ria, 250
m, 317, 318
r, 4
egetables, dietary properties of, 561, 2
in content of (*see Appendix*)
n (pea), 105
, 15, 410
in content of (*see Appendix*)
lo da Vinci, 2

Leptothrix, 618
Lesions, nerve, in vitamin A deficiency, 317
skin, in vitamin A deficiency, 309
Leucine, 105, 238
formula of, 84
indispensable in nutrition, 107
Liebig, 8
"Line Test", 355
Linoleic acid, value of, in nutrition, 27, 36, 57-60
Linolenic acid, value of, in nutrition, 27, 57-60
Lipids (*see* Fats)
Liver, dietary properties of, 139, 142
effect on egg white toxicity, 150
in metabolism of vitamin A, 328
in pernicious anemia, 236
injury to, from amino acid imbalance, 144
vitamin content of (*see Appendix*)
Longevity, effect of dietary protein level on, 136
through dietary improvement, 635-637
through retardation of growth rate, 633-635
Lumiflavin, 485-488
Lumisterol, 341, 342, 350
Lunin, 16
Lysine, 95, 96, 102, 104-106, 139-141, 144
formula of, 85
indispensable in nutrition, 21, 109
replaceability of, by related compounds, 109
requirement of rats for, 109

Magendie, 6, 15
Magnesium, biological availability of, 164
content of, in foodstuffs, 187
content of, in the body, 156, 157
effect of deficiency of, 27, 161-163
effect on calcium and phosphorus metabolism, 175, 176
human requirement for, 186
mobility of, in tissues, 180, 181
state of, in foodstuffs, 164
Maize, cryptoxanthin in, 553
cultivation of, 557
dietary properties, 21, 131-133, 138, 557, 558
vitamin content of (*see Appendix*)
Malic acid, 47
Mammary gland, metabolism of fats in, 74, 75
Manganese, distribution of, 262
effects of calcium and phosphorus on metabolism of, 168, 266
effects of deficiency in diet, 28, 262-267

Manganese, effects of deficiency on hatchability of eggs, 266
effects of large amounts of, in diet, 267
estrus cycle in deficiency of, 262-264
human requirements for, 267
lactation in deficiency of, 262, 263
perosis, relation to deficiency of, 28, 265-267
testicular degeneration in deficiency of, 263
McCollum, early studies in nutrition, 19-21, 23
Meat, dietary properties of,
glandular organs, 131, 565
muscle, 130-132, 218, 565
vitamin content of (*see Appendix*)
Menstruation, effect on content of blood hemoglobin, 232
effect on human iron requirement, 231, 232
Metaphosphoric acid, 167, 406, 408
Methionine, 105, 106
factors affecting utilization of, 113
formula of, 85, 112
indispensability in nutrition, 112
relation to cystine, 112
replaceability of, by related compounds, 113
Methyl cysteine, 112
Methyl glyoxal, 465, 466
Methylcholanthrene, relation to vitamin D, 358, 360
Methylene blue, 409
Mice, 411, 491
anemia in, 217
ascorbic acid requirement of, 411
magnesium deficiency in, 161
manganese deficiency in, 263
Microorganisms, nutritional value of proteins in, 132
relation to iodine content of soil, 251
Milk, ascorbic acid in, 425, 426
calcium in, 183, 185
dietary deficiencies and production of, 188
dietary properties of, 130, 131, 133, 138, 141, 150, 564, 565
mineral deficiencies in, 212, 213, 224, 263, 264
phosphorus in, 185
vitamin content of (*see Appendix*)
Milk fever, 158, 159, 390
Millet, dietary properties of, 133, 558, 559
Monkey, 411, 491
Monomolecular films, 93, 94
Mottled enamel, 280, 281
Mould, assay for thiamin, 453, 454
nutritive value of proteins in, 132
Mulder, 7
Muscular degeneration, factors f
or prevention of, 529
in Herbivora, 527-529
pathology of, 528
production of, 527, 528
role of diet in, 528
symptomatology of, 528
Muscular dystrophy (*see* Muscular
eration)
Muscular incoordination, in vitamin
ficiency, 316
Myxedema, 248

Nerves, lesions in vitamin A def
317
Nickel, distribution of, 271
Nicotinic acid, 557
chemical properties of, 514
formula of, 513
role in biologic oxidations and
tions, 514
role in blacktongue, 512, 513
role in human pellagra, 512, 513
role in the nutrition of microorga
513
role in the nutrition of the rat,
Nicotinic acid amide, relation to cozy
514
relation to the Warburg-Ch
enzyme, 514
Night-blindness, 290
relation to vitamin A content of
310
Nitrogen balance method, of determ
nutritive value of proteins, 122
Nitrogen equilibrium, 11, 12
Norleucine, formula of, 84
significance in nutrition, 107
Nutrition, biological methods in, 21
concept of deficiency disease, 15-19
early chemical discoveries of basic
nificance to, 3-6
early history of, 1 *et seq.*
early physiological studies of basic
nificance to, 5-9
factors for lower forms of life, 52
of bacteria, 529
present concept of, 29
Nutritional muscular dystrophy (*see*
cular degeneration)
Nutritional (biological) value of pro
120 *et seq.*
deamination on, 141, 142
determination by, growth methods
nitrogen balance method, 122-12
nitrogen retention method, 126
paired feeding methods, 127
effect of heat on, 137-141
ultraviolet light on, 141

nal (biological) value of proteins, cereals, meats, nuts, etc. (*see Appendix*), 130-132
ietary properties of, 132, 561
in content of (*see Appendix*)
l, 168
in content of (*see Appendix*)
ietary properties of, 21, 131, 558
in content of (*see Appendix*)
, copper in ink sac of, 226
blasts, effect of dietary deficiencies , 418-421, 606, 607
in dentin formation, 603, 606, 607
ne (*see* Estrone)
, 216, 227
in content of (*see Appendix*)
c acids, nutritional significance of, -50
, 95, 96
e and Mendel, early studies in nu- tion, 19-21, 633
ation, mechanism of, 178-180
alacia, role of vitamin D in, 387, 388
es, effect on calcium utilization, 165
itional significance of, 48, 49
acid (*see* Oxalates)
is, relation of vitamin A, 316
ion of vitamin B_4, 519
ion to vitamin E deficiency, 543, 44
yroid glands, effect on assimilation calcium and phosphorus, 171, 172
elation to mode of action of vita- in D, 170-172, 374
ony in nutrition, 12
dietary properties of, 559
min content of (*see Appendix*)
aring, 16
a, dietary treatment of, 514, 515
theories of etiology of, 500, 501
y acid factor" in, 508
spirochetal organisms in, 504, 505
rical, 499
ne chick, 26, 510
ne rat, 506-509
diseases in animals, 506-509
gnition of its dietary origin, 501, 2
on of blacktongue to, 503, 504
on of light to, 505, 506
of nicotinic acid in, 512, 513
toms in humans, 500
-preventive factor, 25, 511, 512
an, 588
steine, 112
metabolism, 36, 37
238
Peptides, 87, 100, 101, 167, 238
Pereira, 15
Perlèche, correlation of B_6 deficiency and etiogy of, 508
Pernicious anemia, anti-, factor, 237, 238
Castle theory of etiology of, 237
composition of blood in, 239
condition of bone marrow in, 236
extrinsic factor, 237, 240
intrinsic factor, 237
therapeutic effect of liver, 236
Perosis, relation to manganese deficiency in diet, 28, 265-267
Peter Piper-Jack Spratt illustration, 103
Phaseolin, 140
Phenylalanine, 105, 106
formula of, 85
indispensability in nutrition, 110
Phosphatase, in diagnosis of rickets, 356
relation to bone formation, 356, 370
role in ossification, 179, 180
role of blood phosphatase in rickets, 370
Phospholipids, fatty acids in animal tissues, 66, 67
function of, 67
nutritive significance of phosphorus in, 166
relation to fat metabolism, 62, 63
Phosphopeptone, 167
Phosphoproteins, nutritive significance of, 167
Phosphoric acid esters, synthesis of, in body, 166
Phosphorus, action of vitamin D on utilization of, 170, 171
aphosphorosis in farm animals, 160, 161
biological availability of, 165-168
calcium:phosphorus ratio, 159, 172-174, 185, 219, 220, 371, 372
cations which interfere with utilization of, 168, 169, 266, 277
content in the body, 156
content of, in foodstuffs, 187
effect of acid-base balance on utilization of, 169
effect of fat on absorption of, 165
effects of deficiency of, 159, 160
forms in the body, 157
human requirements for, 185, 186
preparation of diet deficient in, 159
radioactive, as indicator, 181
Phycomyces blakesleeanus, 453
Physiological antagonism, between calcium and magnesium, 175, 176
Physiological economy in nutrition, 12
Phytin, 164, 168
Phytol, 295

Pica, in animals, 160, 161, 575-577
iron eating, 577
Pigments, carotenoids, 295, 303
yellow, relation to vitamin A, 292
Piper methysticum, 590
Plasma protein regeneration, effect of different foods on, 148
relation to dietary protein, 147-149
Plastein, 97
Plexaurella dichotoma, 95
Podolite, 156
Polished rice, deficiencies of, 21
Polycythemia, 215, 219, 220, 267, 270, 426
Polyneuritis, 452, 456, 459, 460, 465, 477, 478
Polyphenol oxidase, 227
Porphyropsin system, 304
Potassium, effects of deficiency in diet, 201-203
relation to adrenal function, 205, 206
relation to Familial Paralysis, 207
relation to intestinal stasis, 207, 208
P-P factor, 441, 511
Prairie dog, ascorbic acid synthesis in, 411
Pregnancy and lactation, 184-186, 232-234, 258, 331, 385, 390, 428, 429, 471, 477
Proline, 105, 106, 238
dispensability in nutrition, 111
formula of, 86
Proteins, Alcock theory of synthesis of, 97
amino acid composition of, 105; variability in, 104
amino acid residues in, 91; relation to physico-chemical and physiological properties of, 92
biological availability in foodstuffs, 128, 129
chemical nature of, 79-94
classification on basis of composition, 5, 94-96
constancy of molecular ratios of basic amino acids in keratins, 95; in orosins, 96
cyclol theory of structure of, 89, 90
early views of, 5, 9, 11, 12, 78, 79
effect of diet on chemical composition of, 102
effect of heat on utilization of, 137-141
effect of roughage on utilization of, 129
effects of different amounts in the diet, 134-137, 142-147
enzymes as specific organizers in synthesis of, 100
enzymes, -nature of, 79
-free milk, 20
human requirement for, 11, 12, 133-135
Proteins, inconstancy of cystine c
keratins, 95; of orosins, 96
monomolecular films of, 93,
nutritional value of, 102-106
pendix)
objectives of nutritional studie
orosin, 95, 96
peptide structure of, 87, 88
plastein, 97
relation to ulcers, 149, 150
sedimentation rate and molecula
of, 89, 90
significance in nutrition, 78 *et*
synthesis of, in living matter, 9
viruses, -nature of, 79, 89, 101
Proteins and amino acids, supple
relationships between, 132, 1
Protein metabolism, conversion int
hydrate, 82
deposit protein in, 83
Folin's theory of, 81, 82
theories of, 79-83
Prothrombin, relation to vitamin K
Prout, 7
Provitamin A, 291, 292
Provitamin D, 339, 340, 349, 35
375
Pseudopregnancy, 540, 546
Psoriasis, role of vitamin D in, 38
Purified diets, early experiments w
15-18
Pyridine derivatives, effect of, in
tongue, 512, 513
Pyridine, relation to oxidation-red
514
Pyruvic acid, 463-465, 469

Quinic acid, 50

Rabbits, ascorbic acid requirement c
goiter in, 256-258
milk anemia in, 213
Rats, ascorbic acid synthesis in, 411
dermatitis factor (*see* Vitamin
growth and reproductive data, 63
life span of, 634, 636
Refection, role of carbohydrate in, 3
role of fat in, 61, 62
Renal calculi (*see* Calculi)
Reproduction, effect of, different le
dietary calcium on, 636
different levels of dietary prote
136
manganese deficiency on, 262-2
potassium deficiency on, 202, 2
sodium deficiency on, 200
vitamin A deficiency on, 322-3
vitamin E deficiency on, 27, 539
547-549

atory quotient, 36
, in vitamin A deficiency, 312
min A in, 303
ne, 303
ne$_2$, 304
natic fever, role of ascorbic acid in, 32, 433
psin system, 304
rb, oxalate in, 48
avin, assay of, biological method, 495
ogical activity of, 494
mical and physical methods of estination, 495
mical and physical properties of, 488, 89
tent of foodstuffs (*see Appendix*)
ciency in the dog, 491, 492
ciency in the rat, 490, 491
ribution of, 496
retion, 493, 494
nula of, 487
ction of, 489
orical development, 483, 484
cataract formation, 491
chick nutrition, 492, 493
oxidation-reduction processes of the ell, 489, 490
pediculosis, 491
ation of, 485
ure of, 489
nenclature, 484
tion to phosphoric ester of, 489
tion to yellow oxidation enzyme, 489
uirement of the chick, 493
uirement of the dog, 492
uirement of the human, 495
uirement of the rat, 492
in pellagra, 493
rage of, 493
thesis of, 485, 486, 488
avin phosphoric ester, 489
ose, 488
dietary properties of, 21, 556
ts, anatomical changes in, 368
gnosis by means of composition of lood, 356
one ash content, 357
"line test", 355
crum phosphatase, 356
"X-ray", 356
tary production of experimental, 354, 355
ct of breast feeding on, 385, 386
logy of, 365-367
tabolism in, 380-382
ation of geographic distribution of, to distribution of ultraviolet light, 378-380
e of blood phosphatase in, 370
Rickets, role of bone phosphatase in, 370
role of calcium in, 370
role of Ca:P ratio in, 371, 372
role of phosphorus in, 370
significance of vitamin D in, 363
structure of bone in, 364
Rock phosphate, fluorine in, 280
Rye, dietary properties of, 558
vitamin content of (*see Appendix*)

Saccharose (*see* Sucrose)
Salicylic acid, 50
Saliva, composition of, 608
relation of, to dental caries, 612, 613
Salmin, 105
Salt (NaCl) craving in man and animals, 192
effects of excess of, 194, 195
iodized, 246, 251-254
tolerance in chickens, 194
Sanctorius, 3
Scurvy (*see* Ascorbic acid)
experimental production of, in animals, 417
Sea water, 250
Seaweed, iodine in, 245, 250, 258
Selenium, absorption of, by plants, 284, 285
poisoning from, 284-286
Self-selection of foods by children, 574
Serine, 105
dispensable in nutrition, 107
formula of, 85
Sex differences, in carbohydrate metabolism, 39, 40
Sex hormones, relation to vitamin D, 358, 359
Silicon, distribution of, 274
Silk fibroin, 91, 95, 105
Sitosterol, 345, 348
Skeleton, calcium, magnesium and phosphorus in, 155, 156
Skin, lesions in vitamin A deficiency, 309
role of, in absorption of vitamin D, 376
vitamin D activity of, 374, 375
Slavic peoples, diet of, 597, 598
Sodium, potassium and chlorine, 192 *et. seq.*
absorption and excretion of, 195
dietary requirement for, 15, 208
distribution of, 194
general functions of, 195, 196
role of sodium and potassium in carbohydrate metabolism, 51, 52, 204, 205
Sodium chloride (*see* Salt), effect of deficiency in diet, 203, 204.
Sodium, effects of deficiency in diet, 193, 196-201
relation to adrenal function, 205, 206

"Soft pork", 71
Soil composition, effect of, on mineral content of plants, 160, 187, 188, 251
l-sorbose, 402, 403
Sorghum, dietary properties of, 558, 559
Soybeans, dietary properties of, 133, 140, 141, 559, 560
effect of heat on nutritive value, 128, 140
vitamin content of (*see Appendix*)
Spallanzani, 6
Spinach, 48, 165, 218
vitamin content of (*see Appendix*)
Sprue, 239, 240
Squalene, 295
Starch, 40, 41
Sterility, relation to, fat-free diets, 56
manganese deficiency, 262-265
vitamin A, 322-324
vitamin E, 538-541, 547-549
Sterols, 297, 341, 342
activation of, 337-339, 341
relation of vitamin D to, 342-348
Stigmasterol, 346, 348
Streptococcus mutans, 612
Strontium, effects of, on bones and osteoid tissue, 274
Succinic acid, 50
Sucrose, 36, 41, 42
Sugar, consumption of, in various countries, 471, 616
in dental caries, 615-617, 628
Sulfur-containing amino acids, effect on tissue growth, 115
relation to epidermal tissues, 113-115
Summer, vitamin D administration in, 378
Sunlight, relation to vitamin D, 24, 337
Swine, 316, 411

Tachysterol, 341, 343, 350
Takaki, 17
Teeth, effect of ascorbic acid deficiency on, 418-421, 624, 625
effect of fluorine on, 279-282
effect of vitamin A deficiency on, 319
mobility of minerals in, 181
Testis, in manganese deficiency, 263
injury of, in vitamin A deficiency, 323, 324
Tetany, grass tetany, 177, 178
role of calcium, magnesium and phosphorus, in, 176, 177
role of vitamin D in, 387
Therapy, vitamin A, 317, 331, 332
in hyperthyroidism, 327
in senile vaginitis, 323
vitamin D, 387-390
Thiamin, anorexia in deficiency of, 463
biological tests for, 451, 452
Thiamin, borderline states of d
473, 474
catatorulin test for, 453
chemical and physical proper
443, 447, 448
chemical nature of, 441 *et*
Cowgill's formula, 470
craving in rats for, 474
effect of alkali on, 448, 4
effect of cooking on stabilit
effect of deficiency of, on bi
ing substances, 466
effect of massive intakes, 46
effects of deficiency of, 459-
electrocardiographic method
ing, 453
extent of deficiency in We
tries, 471-473
factors affecting requiremen
469
fermentation assay for, 454
gastrointestinal functions in
of, 461, 462
heart in deficiency of, 461
human needs for, 469-471
in alcoholic neuritis, 477, 4
in cardiovascular dysfunction
in gastrointestinal disorders,
in pregnancy, 477
in renal injury from excess
protein, 143
International and other uni
451
isolation and identification
443
mould growth method of
453, 454
nervous system in deficiency
nomenclature of, 441, 442
nutritional significance of, 4
nutritive effect of intermedia
physiological role of, 464-46
pyrophosphate (Cocarboxyla
464-466
qualitative tests for, 454, 455
relation to bacterial growth,
relation to cocarboxylase, 44
relation to plant growth, 4
relation to thiochrome, 447,
sparing action of fats on, 46
storage and excretion of, 466,
synthesis of, 443-446
therapeutic uses of, 476
Thiochrome, 447, 448, 454
Threonine, formula of, 85
indispensability in nutrition,
Thyroglobulin, 106, 249
Thyroid gland (*see* Iodine)
activity of, and thiamin nee

land, effect of iodine deficiency
47-249
vitamin E deficiency on, 545
of vitamin A to, 327
formula, 249
rs of, 121
ol (*see* Vitamin E)
ol (*see* Vitamin E)
ol (*see* Vitamin E)
rils, 420, 607
elopment, 602-608
pregnancy, role of vitamin D in,

rganic elements, 261 *et seq.*
nally indispensable, 262
s, role of vitamin D, in, 389
Cunha, 591, 592
eld Zone, 421
ne, 98-100, 102, 104-106, 139-
144
of, 85
nsability in nutrition, 21, 110
bility of, by related compounds,

d root vegetables, dietary prop-
of, 560
sis, role of ascorbic acid in, 433,

relation of vitamin E to, 546
227
102, 104-106, 139, 141, 144
ability in nutrition, 111
a of, 85

rat "pellagra", 507
of, to low-protein diets, 149,

t light, effect of, on proteins,

of, to geographic distribution of
ts, 378-380
of various sources of, to vitamin
, 337, 338
rnational, of ascorbic acid, 410
nin, 450
nin A, 304
nin D, 357
s, relation to vitamin A, 321,

99.

5
of, 84
sable in nutrition, 107
, 275
ism, 563, 564
, 142
in pernicious anemia, 238

Viruses, in pellagra, 504, 505
protein-nature of, 79, 89, 101
Visual mechanism, 303, 496
Visual purple, 303
in night blindness, 311, 312
in vitamin A deficiency, 312
regeneration of, 311
relation to vitamin A, 311, 312
synthesis of, 303
Visual white, 303
Visual yellow, 303
Vitamin A, absorption of, 327, 328
assay of, 304-306
biological activity of, 303
chemical nature of, 290-306
deficiency of, 290, 291, 312
distribution of, 306
early history of, 19, 20
effects of massive doses of, 331
excretion of, 329
functions of, 327
International unit of, 304
isolation of, 298
mammary transmission of, 330
normal requirements of, 330, 331
nutritional significance of, 308-332
properties of, 298-300
relation of yellow pigment to, 291, 292
relation to artificially induced infections, 325, 326
relation to infection, 324, 325
requirements in disease, 328, 331
role in healing of wounds, 327
role in thyroid function, 327
role in visual mechanism, 303
storage of, 329, 330
structure, 294, 301, 302
synthesis, 300-303
therapy, 323, 327, 331, 332
uterine transmission of, 330
Vitamin A deficiency, 290, 291, 312
biliary obstruction in, 128
effect on bone, 318, 319
effect on epithelial tissues, 313-315
effect on estrus cycle, 322
effect on growth, 308, 318
effect on the hematopoietic system, 319, 320
effect on nervous system, 315-318
effect on reproductive function, 322-324
effect on seminiferous epithelium, 323, 324
effect on teeth, 319
effect on visual purple, 311, 312
hemeralopia, 309
immunological reaction in, 313-315, 326

Vitamin A deficiency, keratinization in, 313, 314, 315, 316
keratomalacia, 309, 310
metabolism in, 320, 321
muscular incoordination and, 316
night blindness and, 310
skin lesions in, 309
testicular degeneration in, 323, 324
urinary calculi in, 310, 321, 322
visual disturbances in, 311-312
xerophthalmia and, 310, 317
Vitamin A_2, 304
Vitamin B complex, 217, 239
discovery of multiple nature of, 441
early studies of, 24, 25
effects of supplements of, on infants and children, 474-476
in preventing renal injury, 143
nomenclature of, 441, 442
Vitamin B_1 (*see* Thiamin)
Vitamin B_3, 518
Vitamin B_4, 518
deficiency of, in the chick, 519
deficiency of, in the rat, 518, 519
effect of, on chick paralysis, 519
occurrence, 519
Vitamin B_5, 519
Vitamin B_6, chemical and physical properties of, 510
deficiency of, 508
formula of, 510
functions of, 509
isolation of, 509
relation of perlèche to deficiency of, 508
role in microcytic hypochromic anemia in puppies, 509
role in rat pellagra, 506-509
Vitamin C (*see* Ascorbic acid)
Vitamin D, absorption, 377
absorption spectra of, 339, 340
activity of skin, hair, wool and feathers, 374, 375
administration of, during summer, 378
chemical nature of, 336
distribution of, in foods, 352, 353
early views of deficiency of, 336, 337
effect of, on blood coagulation, 390
effect of, on milk fever, 390
effect of various sources of, on humans, 383, 384
effects of excessive dosage of, 386, 387
excretion, 377
formation of, 337-341
forms of, 328, 347, 348
formulae of, 343, 344, 345
in nutrition of the chick, 340
in nutrition of the rat, 340
mammary transmission of, 377, 378
natural, 348, 349
Vitamin D, origin of, in animals
origin of, in plants, 351
physical-chemical properties of,
relation of other sources of
to, 24, 338
relation of sunlight to, 24, 33
relation to cancerigenic substan
359
relation to dental caries, 391-3
relation to linear growth, 382,
relation to parathyroids, 171,
relation to sex hormones, 358,
relation to sterols, 342-348
requirements for, in lactation,
in pregnancy, 385
in rickets, 385
normal, 384
role in calcium and phosphorus
tion, 170, 171
role in development of embryo,
role in diseases related to ricket
role in fracture healing, 391
role in infantile paralysis, 390,
role in mineral metabolism, 37
role in osteomalacia, 387, 388
role in rickets, 363
role in tetany, 387
role in wound healing, 391
storage, 376
therapy, 387-390
unit of, 357
Vitamin D deficiency (*see* Rickets)
early views of, 23, 24, 336, 33
effects on fecal calcium and pho
170
effects on fecal reaction, 170
eye changes in, 389, 390
Vitamin D_2 (calciferol), 349, 350
Vitamin D_3, 350
Vitamin D_4, 350, 351
Vitamin D milk, 353, 354
assay of, 354-357
Vitamin E, 532 *et seq.*
allophanates of, 534, 535
and antioxidants, 532-534
assay methods for, 546, 547
chemical and physical properties
535
chemical structure and synthesis
538
content of various forms of, in
destruction of, by ferric chloride,
destruction of, by rancid fats, 53
distribution of, 547
durohydroquinone from, 536
early observations on nutritive
cance, of, 26, 532, 538, 539
effect of deficiency of, on growt
543

, effect of deficiency of, on
bility of egg, 542
f deficiency of, in female, 27,
40
f deficiency of, in male, 27,
41
ular dystrophy, 529
of, 534
from deficiency of, 543, 544
gical activity of synthetic α
erol, 538
to internal secretions, 545
ent of different species, 547
tic applications of, 547-549
, 60
(*see* Riboflavin)
l, 520
, chemical properties of, 523
y of, 522
of, 523
nce of, 523
to prothrombin content, 522
blood coagulation, 522
hemophilia, 523
nutrition of chicks, 522
nutrition of other species, 522
matology of, 522
s by bacterial action, 522
ssion of, 522
P, 414, 415
19
105, 106
9, 15

-Christian's enzyme, 484, 485,
489
mino acid content of, 104
properties of, 21, 132, 138, 218,
555
es of milling, 554

Wheat, vitamin content of (*see Appendix*)
Wheat germ, dietary properties of, 21, 138, 475, 556
vitamin content of (*see Appendix*)
Wheat germ oil, 142, 533-535, 546-548
Wheat gluten, nutritional value of proteins in, 138
Wool, antiricketic activity of, 375
growth of, in relation to cystine and methionine, 113-115
Wounds, role of vitamin A in healing of, 327
role of vitamin D in healing of, 391

Xanthophyll, 291
Xerophthalmia, 290, 308, 310, 317
"X-ray," of bones in ascorbic acid deficiency, 421, 422
method for determining vitamin D activity, 356
l-xylose, 401

Yeast, 142, 148, 150, 188, 218, 468
amino acids in, 106
nutritional value of proteins in, 132
vitamin content of (*see Appendix*)
Yellow enzyme, 484, 485, 488, 489, 514
Yellow pigment, relation to vitamin A, 291, 292

Zein, 121
deficiency in lysine and tryptophane, 103
Zinc, content of, in crystalline insulin, 268
distribution of, 268
effect of, in perosis, 266
evidences of nutritive indispensability, 28, 268
relation of, to hormonal functions, 269

C MA 9 9 50
MA 14 (4) 15
C
CHECKED
2-5-97
CFTRI-MYSORE
126
Never knowledge...

CHECKED
2008

VERIFIED
2013

CFTRI LIBRARY
B126